THE TOXICOLOGY OF CYCLOTRIMETHYLENETRINITRAMINE (RDX) AND CYCLOTETRAMETHYLENETETRANITRAMINE (HMX) SOLUTIONS IN DIMETHYLSULFOXIDE (DMSO), CYCLOHEXANONE, AND ACETONE

EDGEWOOD ARSENAL

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A study of the toxicology of the explosives cyclotrimethylenetrinitramine (RDX) and cyclotetramethylenetetranitramine (HMX) in acetone, cyclohexanone, and pure and technical grade dimethylsulfoxide (DMSO) was initiated to establish whether there is any danger to plant personnel that handle such mixtures. This report contains a review of the existing literature on each explosive and on each solvent. It also describes tests that were conducted to establish the intravenous toxicity of the explosives in DMSO, the skin effects, the pharmacological effects, the sensitization potential,

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and the ocular effects of the explosives in each solvent. All of these tests were conducted on animals. In mice, the intravenous LD50 for RDX in DMSO is 18.7 mg/kg and for HMX in DMSO in 28.9 mg/kg. In guinea pigs, the intravenous LD50 for RDX in DMSO is 25.1 mg/kg and for HMX in DMSO is 28.2 mg/kg. The LD50's of RDX and HMX in other solvents were not established. RDX and HMX in the three solvents did not penetrate the skin, as evidenced by the lack of physiological responses in dogs and unchanged blood component values in rabbits. From the intravenous studies in dogs, it was shown that acetone and cyclohexanone alone exert a depressant effect on the cardiovascular system. Cyclohexanone also causes changes in the electroencephalogram pattern and produces a semicomatose to comatose condition. DMSO had relatively little effect. Therefore, the majority of these studies were done with the explosives in DMSO. It was found that the immediate effects of RDX and HMX differ, RDX affecting the CNS immediately and HMX producing a circulatory collapse initially, with delayed CNS disturbances. Topically and intradermally applied RDX and HMX in the three solvents did not produce usually any greater skin damage than the solvents alone, but there were several exceptions. Repeated topical applications caused dermatitis without fissures, eschars or scabs, but intradermal injection caused severe skin damage. Topical or intradermal application of the solvents or of RDX and HMX in the solvents 3 days a week for 3 weeks, followed in 2 weeks by topical or intradermal challenge, gave no evidence of sensitization. Ocular administration showed that RDX and HMX are no more damaging than the solvents alone, but the solvents themselves cause cataracts in guinea pigs. From these studies, it is evident that strict precautions should be taken to avoid skin and ocular contact with HMX and RDX in the solvents studied.

SUMMARY

A study of the toxicology of the explosives cyclotrimethylenetrinitramine (RDX) and cyclotetramethylenetetranitramine (HMX) in acetone, cyclohexanone, and pure and technical grade dimethylsulfoxide (DMSO) was initiated to establish whether there is any danger to plant personnel that handle such mixtures.

This report contains a review of the existing literature on each explosive and on each solvent. It also describes tests that were conducted to establish the intravenous toxicity of the explosives in DMSO, the skin effects, the pharmacological effects, the sensitization potential, and the ocular effects of the explosives in each solvent. All of these tests were conducted on animals.

In mice, the intravenous LD50 for RDX in DMSO is 18.7 mg/kg and for HMX in DMSO is 28.9 mg/kg. In guinea pigs, the intravenous LD50 for RDX in DMSO is 25.1 mg/kg and for HMX in DMSO is 28.2 mg/kg. The LD50's of RDX and HMX in other solvents were not established.

RDX and HMX in the three solvents did not penetrate the skin, as evidenced by the lack of physiological responses in dogs and unchanged blood component values in rabbits.

From the intravenous studies in dogs, it was shown that acetone and cyclohexanone alone exert a depressant effect on the cardiovascular system. Cyclohexanone also causes changes in the electroencephalogram pattern and produces a semicomatose to comatose condition. DMSO had relatively little effect. Therefore, the majority of these studies were done with the explosives in DMSO. It was found that the immediate effects of RDX and HMX differ, RDX affecting the CNS immediately and HMX producing a circulatory collapse initially, with delayed CNS disturbances.

Topically and intradermally applied RDX and HMX in the three solvents did not produce usually any greater skin damage than the solvent alone but there were several exceptions. The animals treated with single or multiple 1.0-ml doses of RDX in DMSO consistently had dermatitis while those receiving the same doses of DMSO alone did not. Some of the rabbits that received single or multiple doses of RDX in acetone or cyclohexanone had dermatitis and the solvent controls did not. HMX in acetone and in cyclohexanone, applied repeatedly, caused dermatitis but the solvents alone did not. Repeated topical applications of the mixtures caused dermatitis without fissures, eschars, or scabs, but intradermal injection caused severe skin damage.

Topical or intradermal application of the solvents or of RDX and HMX in the solvents 3 days a week for 3 weeks, followed in 2 weeks by topical or intradermal challenge, gave no evidence of sensitization.

Ocular administration showed that RDX and HMX are no more damaging than the solvents alone, but the solvents themselves cause cataracts in guinea pigs.

From these studies, it is evident that strict precautions should be taken to avoid skin and ocular contact with HMX and RDX in the solvent studies.

PREFACE

The work described in this report was requested by Picatinny Arsenal and authorized by Customer Order Number RA02850202GGF4. The work was started in March 1970 and completed in September 1970. Experimental data are contained in notebooks MN 2242, MN 2130, and MN 2377.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences – National Research Council.

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I. INTRODUCTION.

The object of this report is to present the results of a toxicological study performed at Edgewood Arsenal of the explosives cyclotrimethylenetrinitramine (RDX) and cyclotetramethylenetetranitramine (HMX) dissolved in dimethylsulfoxide (DMS), cyclohexanone, and acetone.

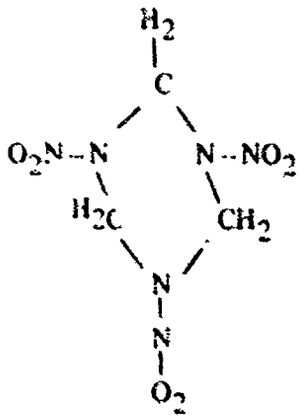
In September 1969 the Toxicology Division was asked by Picatinny Arsenal to perform the study because of concern for the safety of plant personnel who handle RDX and HMX in various solvents. A proposal to conduct single and repeated dose tests of rabbit skin, sensitization tests in guinea pigs, and mechanism of action studies in dogs was approved. In addition to this work, the intravenous toxicity of the compounds was tested in mice and guinea pigs; eye effects in guinea pigs were evaluated; and an extensive review of the literature was prepared.

II. REVIEW OF LITERATURE.

A. RDX.

1. Chemical and Physical Characteristics.[1,2,3]

The formula for RDX is:



RDX is a solid having a melting point of 200-203°C. It is insoluble in water, ether, and carbon tetrachloride, but soluble in some organic solvents such as dimethylsulfoxide (DMSO), acetone, cyclohexanone, hot aniline, phenol, and nitrobenzene.

2. Biological Effects.

The most prominent effect of RDX appears to be central nervous system excitation. Thus, RDX is pharmacologically different from nitrites and nitrates, which act on the cardiovascular system, causing hypotension.

Sunderman, *et al*[4] showed that the acute oral LD50 for nonfasting rats was about 200 mg/kg and for fasting animals was 50-100 mg/kg. When they gave pentobarbital to rats the mortality from a given dose of RDX decreased. RDX given in intravenous doses of 18 mg/kg and intraperitoneal doses of 10 mg/kg killed rats.

The oral LD50 for mice was reported by Slanskaya and Pozharsky[5] to be about 500 mg/kg. They found a dose of 100 mg/kg caused no signs in mice but was lethal to cats.

Patty[1] showed that daily oral doses of 15, 50, and 100 mg/kg for 10 weeks killed 1, 17, and 15 rats, respectively, in groups of 35 animals. The animals became irritable and vicious, and convulsed frequently. The lungs and gastrointestinal tracts of the dead animals were congested. There were no pathological changes in the survivors.

In the experiments of von Oettingen *et al.*[3] one of seven dogs that were fed 50 mg/kg/day of RDX, 6 days/week for 6 weeks, died. The animals became excited and irritable within a few hours after the first dose. Within 1 week, reflexes were hyperactive, salivation was evident, and convulsions and collapse occurred. After the first week, the animals lost weight even though their appetites were good. There were no blood changes, no methemoglobinemia, and no microscopic pathological changes. After acute exposure of animals, Slanskaya and Pozharsky[5] noted changes in the fibrous material of the walls of the blood vessels in the central nervous system and degeneration of the nerve cells; after chronic exposure, the liver, lungs, and heart were also affected.

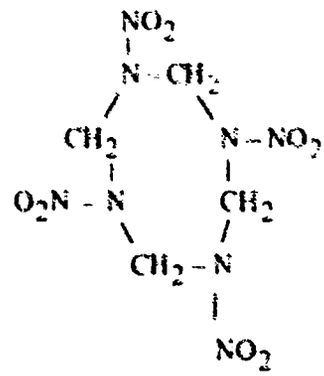
Workers in RDX plants in Italy developed epileptic-like seizures; followed by amnesia, weakness, fatigue, and malaise.[6] These effects occurred most frequently in men performing tasks where inhalation of the dust was possible. Recovery was complete when the individuals were removed from contact with the compound.

In other plants where the RDX was handled in a moist state, systemic toxicity was not seen. Primary irritant dermatitis and skin sensitization did occur during the nitration process. This was possibly caused by unidentified component of the fumes emanating from the reaction mixture.[7,8] The final purified material did not produce dermatitis.[7] Patch tests with moistened RDX did not cause irritation.[3]

B. HMX.

1. Chemical and Physical Characteristics.[9]

The formula for HMX is:



HMX is a solid having a melting point of 279°C. It is soluble in some organic solvents such as DMSO, acetone,[9] and cyclohexanone.

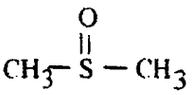
2. Biological Effects.

No information was available from the published literature on the biological actions of HMX.

C. DMSO.

1. Chemical and Physical Characteristics.

DMSO is a colorless liquid with the following formula:



DMSO has a molecular weight of 78.13, a boiling point of 189°C, a freezing point of 18.55°C, and a density of 1.0958 gm/ml at 25°C. It is miscible with water and many organic solvents. It has been widely used for its solvent properties.[10,11]

2. Biological Effects.

DMSO has been studied extensively. It has been used as a skin penetrant and vehicle to carry drugs through the skin.[12] It protects a variety of cells from the damaging effects of freezing and thawing. When present before and during exposure, DMSO protects against radiation damage to cells and whole mammals.[13] It is anti-inflammatory, analgesic, bacteriostatic, diuretic, sedative, and fibrinolytic. DMSO has been reported to be helpful in rheumatoid arthritis, inflammatory conditions, neuroskeletal injuries, and scleroderma. It is beneficial in dermatological, urological, and ophthalmological disorders as well as in diseases of the ear, nose, and throat.[12]

A number of undesirable toxicological effects have also been reported.[11,12] DMSO is metabolized to form dimethyl sulfide, which gives the breath a garlic-like odor. Occasional allergic reactions have been noted. Topical application has caused local and general dermatitis. Prolonged administration of large oral or dermal doses produces lens opacities in swine, dogs, and rabbits.[14-18] Teratogenic effects have also been reported.[19] DMSO does not sensitize the skin of guinea pigs,[20] and does not seem to be carcinogenic.[21,22]

3. Toxicology of DMSO.

a. Single Doses.

There is a close similarity of the signs produced by DMSO in various animal species and by various routes of administration. For example, the LD50's for single, intravenous injections in-

mice, rats, cats, and dogs ranged from 2.5 to 8.9 gm/kg.[18] The signs observed at lethal or near lethal doses were similar in all species (decreased activity, tremors, weakness, dyspnea, stupor, convulsions, hypothermia, and hematuria).[18] However, Fennman *et al.*[23] infused 4.0 gm/kg intravenously into two monkeys without causing death or signs.

Single oral doses also produced similar signs in various species. The signs were like those seen in the intravenous studies except that emesis was also noted.[18]

Subcutaneous and intramuscular injections caused local areas of inflammation, edema, hemorrhage, and necrosis.[18]

DMSO applied to the skin produced transient erythema. When the bodies of mice and rats were immersed in DMSO, most animals died within 24 hours.[18]

Ocular administration produced lacrimation and edema and erythema of the orbital tissues.[18]

LD50 values and toxicity of DMSO by various routes of administration in several animal species are shown in table I as reported by Smith *et al.*[18] and in table II as reported by Caujolle *et al.*[19]

Table I. Single-Dose Toxicity of DMSO

Species	LD50				
	Intravenous	Oral	Subcutaneous	Intraperitoneal	Dermal
	gm/kg				
Mouse	3.8 -- 8.9 (18,24,25,27,28)*	16.5 – 24.6 (18,25,28)	13.9 – 20.5 (18,24)	14.7 – 17.7 (18,27)	⩽50 (31)
Rat	5.2 – 8.1 (18,24,25)	17.4 – 28.3 (18,25)	12.0– 20.5 (18,24)	13.0 (18)	⩽40 (18)
Guinea Pig	–	>11 (29)	–	>55 (29)	–
Chicken		~14 (29)	–	–	–
Cat	~4.0	–	–	–	–
Dog	~2.5 (24)	>10	–	–	>11 (18)
Monkey	>4 (23)	>4 (23)	–	–	>11 (18)

* The numbers in parentheses are references.

Table II. Toxicity of DMSO in Mice and Rats

Effect (24 hr)	Dose			
	Intravenous	Intraperitoneal	Subcutaneous	Oral
	gm/kg			
Mice				
MDNF [a]	5.0	14.0	12.2	⩾14.0
LD50	11.0	20.1	16.0	N.E. [b]
MDAF [a]	14.8	>22.1	22.5	14.0
Rats				
MDNF	⩾ 6.0	11.1	10.0	⩾15.0
LD50	N.E.	13.7	13.7	N.E.
MDAF	> 6.0	15.0	15.0	>15.0

[a] MDNF – maximum dose never fatal
MDAF - minimum dose always fatal

[b] N.E. - not evaluated

b. Repeated Doses.

(1) Intravenous.

The intravenous LD50 DMSO for dogs is about 2.5 gm/kg.[24] Rosenkrantz *et al.*[24] gave two dogs steadily increased intravenous doses of DMSO daily. They survived 1.86 gm/kg but died when dose was increased to 2.95 gm/kg.

Intravenous doses of 0.3 to 2.4 gm/kg of DMSO (both undiluted and diluted) were given to dogs by Willson *et al.*[25] 6 days per week for 4 weeks. Anemia, hemoglobinuria, bilirubinuria, increased serum glutamic oxalacetic transaminase activity, iron-positive pigment in the liver, spleen, and kidney, as well as cloudy swelling in the liver resulted. The four dogs that received the 2.4 gm/kg dose died.[25]

(2) Oral.

Oral doses of 2.5 gm/kg/day given by Caujolle and coworkers (cited by Smith *et al.*[18]) for 6 weeks to mice caused degeneration of the liver and indications of nephritis. When Caujolle gave oral doses of 1, 2 and 5 gm/kg/day for 6 weeks to rats, these doses also caused changes in the liver and kidneys. Smith[18] (unpublished data) found reduction in body weight, and some organ

weights, but no gross adverse effects when 1, 3, and 10 gm/kg were administered orally to rats for 59 consecutive days. Rats survived oral doses of 0.4 and 7.0 gm/kg for 13 weeks in the experiments of Sommer and Tauberger (cited by Smith *et al.*[18]). The lower doses caused some atrophy of the spleen. Doses of 14.1 gm/kg caused sedation and death. Hematological findings and urinalysis were normal. Necropsy revealed generalized hyperemia, gastrointestinal hemorrhage, and splenic changes.

Smith *et al.*[18] reported that oral doses between 2.5 and 10 gm/kg given for 14 days caused death in one of three dogs. Continued dosing of the other two animals caused sedation, ulceration of the oral mucosa, injected sclera, muscle tremors, elevated hemoglobin and hematocrit, increase in transaminase activity, fatty degeneration of the liver, and hemorrhages in the gastro-intestinal tract. These doses also caused some changes in the ocular lens after 48 days.

Monkeys tolerated five daily oral doses of 4.0 gm/kg without adverse effects.[23]

Daily oral administration of 1 or 3 ml/kg, or dermal administration of 1, 3, or 9 ml/kg of DMSO to rhesus monkeys for 18 months produced no toxicological effects in body weight, blood pressure, heart rate, respiratory rate, body temperature, water consumption, neurological reflexes, electrocardiograms, hematology, and urinary constituents.[26] There were no pathological changes or lenticular effects attributable to DMSO. Some animals receiving oral doses of 9 ml/kg died between 15 and 53 days of study. Atelectasis and emphysema were the only pathological changes.

Repeated doses of DMSO that did not produce deaths, as reported by Smith *et al.*[18] are shown in table III.

Table III. Highest Doses of DMSO Not Producing Deaths When Administered 3-7 Days Per Week for 2-26 Weeks

Species	Dose				
	Intravenous	Oral	Subcutaneous	Intraperitoneal	Dermal
	gm/kg				
Rat	2.5 (24)*	11.0 (29)	10.0 (24)	8.2 (29)	10 (18)
Dog	4.8 (18)	10.0	–	–	11
Monkey	4.0 (23)	4.0 (23)	–	–	11.0 (18)

* The numbers in parentheses are references.

c. Effects on Skin.

(1) Animals.

Brown *et al.*[29] painted neat DMSO on hairless mice twice a week for 30 weeks with no noticeable effects. They also found no gross or microscopic signs of damage when the liquid was applied to clipped backs of guinea pigs daily for 28 days.

Undiluted DMSO and 60% solutions were applied to the shaved skin of dogs and monkeys by Smith *et al.*[18] in doses of 3.3 to 33.0 gm/kg/week for 6 months.[18] Application produced transient warmth and reddening of the skin. Furfuraceous and membranous desquamation of the epidermis started within 3 weeks and persisted throughout the experiment but microscopic examination revealed no involvement of the deeper layers. Cutaneous application to rats produced hyperkeratosis, parakeratosis, and focal ulceration.[18]

When applied topically to anesthetized dogs, increased temperature of the skin, subcutaneous tissues, and underlying muscles was noted by Bradham and Sample.[30] DMSO did not produce dermal sensitization in guinea pigs tested by Goldman *et al.*[20]

(2) Man.

Kilgman[31] applied 9 ml of 90% DMSO to the torsos of 20 people for 26 weeks. He noted a transient erythema, mild scaling, and diffuse erythematous dermatitis. Skin biopsies of other subjects who received twice as much DMSO showed a mild perivascular lymphocytic infiltration, acanthosis, absence of a granular layer, and parakeratosis.

Various investigators have described dermal effects of DMSO. Erythema[32] heat,[20,33] local irritation,[20,34,35] burning and tingling[36,37] are often noted immediately or soon after application. The burning sensation lasts 10 to 30 minutes,[36] and the erythema disappears within 1 hour.[32] These effects are less intense with continued use.

With repeated use and occasionally after a single application, a variety of skin effects are noted.[32] These include redness, rashes,[36,38,39] peeling,[36] scaling,[32,36] local dermatitis,[39-41] general dermatitis,[32] and vesiculation.[32,36] The site of application sometimes becomes sensitive to sunlight.[40]

Sulzberger[42] reported on wheals and flares following application to scratches or intracutaneous injection. DMSO may cause liberation of histamine.[38]

Urticaria,[20,35] angioneurotic edema, and swelling of the tongue have been mentioned as consequences of using DMSO.[35] Bad breath is a frequently mentioned effect noted after dermal application.[32,34,36,37,39]

(d) Ocular Effects.

The ocular effects of DMSO require special mention since these actions prompted the Food and Drug Administration to ban human testing for a time.[43] Rubin and Barnett[14] found lens changes in dogs after 9 weeks of oral dosing at 5 gm/kg/day. Doses of 2.5 gm/kg/day caused opacities after a longer period. The dogs were refractory to the mydriatic effect of tropamide. Lenticular changes occurred in 90 days following dermal doses of 4 ml/kg/day in rabbits of 4.5 ml (90% DMSO)/kg/day in swine.

Wood *et al.*[15] in their studies of rabbit eyes found that oral doses of 8 to 11 gm/kg/day produced hazy lenses in 1 to 2 weeks. Topical application of 10 gm/kg/day on the back also causes some haziness. Hematological findings in these animals were normal. Single drops of 10, 15, 30, and 100% DMSO in rabbit eyes three times/day for 6 months caused no effects except lacrimation. Animal growth and hematological and pathological findings remained normal.

When Kleberger[16] administered 9 ml/kg/day (50% DMSO) to beagle dogs by stomach tube, it caused vomiting. Marked refraction changes toward myopia were noted in 1 week. A slight opalescence of the lens developed after 2 months.

Lenticular changes and myopia are not readily seen upon ophthalmoscopic gross, or microscopic examination of the lens. Examination by retinoscope or slit-lamp is required. Smith *et al.*[17] found lenticular changes and myopia in dogs after oral administration of 5 or 10 gm/kg/day of DMSO. Without slit-lamp examination, no abnormalities were noted in dogs following intravenous doses of 1.6 gm/kg/day for 62 days, or in one dog that was given 2.4 gm/kg/day for 33 days, followed by 4.8 gm/kg/day for 28 days. Dermal doses of 6.6 gm/kg given 5 days/week for 6 months did not reveal any abnormalities.

Barnett and Noll[44] found changes in the refractive index of the crystalline lens of seven out of eight rhesus monkeys receiving 9 ml/kg/day orally and suspected such changes in six of eight animals dosed with 3 ml/kg/day. An increased brightness first appeared at the central or nuclear zone of the lens which later became clearly demarcated from the peripheral zone. However, these changes were not seen in monkeys receiving intravenous infusions of 4 gm/kg/day 5 days a week for 69 days,[23] oral doses of 5 gm/kg/day for 100 days,[45] topical application up to 33 gm/kg/week for 6 months,[18] or following daily doses for 18 months of 1 or 3 ml/kg orally or 1,3, or 9 ml/kg dermally.[26]

Gordon[46] reported that a 16-year old boy with severe bilateral uveitis exhibited a small subcapsular lens opacity for approximately 5 months of treatment with eye drops of DMSO. The opacity was not increased with continued administration of DMSO and was believed to be a consequence of the uveitis and not the therapy. Other patients received the eye drops for 1 to 15 months.[46]

Numerous studies in man have not revealed any actions on the lens.[32,35,37,39,40,41,46]

c. Teratogenic Effects.

In 1965, Caujolle *et al.*[47] found that low doses of DMSO in different species produced some abortions but no malformations. In 1966, these investigators[19] gave oral or intraperitoneal doses of 5 to 12 gm/kg to mice during the 6th to 12th day of gestation. Doses of 5 to 10 gm/kg were given to rats by the same route and for the same time course. The animals were sacrificed 1 to 3 days before parturition. DMSO produced malformations in the mice after the intraperitoneal doses, but not after oral administration. Both oral and intraperitoneal doses caused abortion and malformations in the rats.

Rabbits received 5 gm/kg orally or 4 gm/kg subcutaneously from the 6th to the 14th day of gestation.[19] The animals were sacrificed on the 20th to 24th day of gestation. Only one malformation was found among 83 fetuses.

Malformations were produced in the chick embryo when doses approaching the LD50 (10.3 mg/embryo at 72 hours and 12.2 mg/embryo at 96 hours) were employed.[19]

Ferm[48] found DMSO to be teratogenic when administered in high doses to hamsters.

A single oral dose (10 ml/kg) of DMSO given during the period of organogenesis (7th day of gestation) was teratogenic to hamsters.[49]

f. Tumorigenic Actions.

Research in the area indicates that DMSO is not tumorigenic. The influence of the daily ingestion of 50 ppm of DMSO on the production of breast tumors was studied in Sprague-Dawley rats. The DMSO was started 3 days before or after gavage with 7,12-dimethylbenz[*a*]anthracene. The gavage alone produced tumors in almost all animals in 4 to 8 weeks. After 18 months DMSO had no beneficial or deleterious effect on the number of rats that developed tumors, or on mortality. Rats that received DMSO had fewer tumors than control animals.[21]

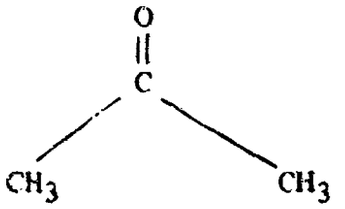
DMSO was more cytocidal to leukemic lymphocytes than to normal lymphocytes. Cells from patients with acute granulocytic leukemia, cells of spontaneous lymphomas of AKR mice, and cells of transplantable rat lymphoma also were relatively sensitive to DMSO.[22]

DMSO was found to be of value in research on cancer of the cervix. When Ayre and LeGuerrier[50] applied the compound to precancerous lesions or to *in situ* carcinomas, no structural changes were seen. When decardron, an adrenal steroid, was applied in DMSO to such tissue, regressive cytological changes occurred in 2 to 3 weeks. Barium chloride changed normal epithelial cells of the cervix to bizarre multinucleated cells characteristic of premalignant dysplasia. DMSO potentiated this effect of barium chloride.

D. Acetone.

1. Chemical and Physical Characteristics.

Acetone (2-propanone; dimethyl ketone) is a colorless liquid with the following formula:



It has a molecular weight of 58.08, a boiling point of 56.1°C, a freezing point of -95.6°C and a density of 0.7911 gm/ml at 20°C. It is miscible with water and other organic solvents.[51,52]

It is widely used as a solvent for resins, fats, oils, collodion cotton, celluloid, cellulose acetate, etc. It is also used in the manufacture of smokeless powder, explosives, lacquer, varnishes, celluloid products, rubber compounds, chloroform, ketones, iodoform, sulphonol, dyes, cements, adhesives, artificial silk, artificial leather, and lubricating oils.[53]

Acetone is prepared commercially by the destructive distillation of wood, by distillation of calcium acetate, by fermentation of corn products using selected bacteria, and by catalytic oxidation of isopropyl alcohol, cumene, or natural gas.[52]

Because of its low flash point, the fire and explosive hazard of acetone is a major factor in its handling.[52]

2. Hazard and Physiological Considerations.

Acetone has a more rapid narcotic action than methyl alcohol when received intravenously, orally, or by inhalation, but is less toxic. Its effect is similar to but greater than that of ethyl alcohol. Due to its volatility the most likely route of administration is by inhalation. When inhaled, acetone is readily absorbed in the blood stream because of its solubility in water and thus transported rapidly throughout the body.[53] Eye and skin contact may occur, but ingestion is not likely because of its sharp and bitter taste.[52] When absorbed through the skin, acetone penetrates more slowly than ethyl ether or chloroform because its high solubility in water results in slow penetration through the epidermal cells.[54] The danger of skin absorption is small and unlikely to occur in normal industrial operations.[53] The principal hazards to health are directly related to the inhalation of vapors at very high concentrations and to repeated and prolonged extensive skin contacts because of the potent solvent effects of the compound.[52] Acetone also has an irritating effect on mucous membranes.[53]

Some early investigators considered acetone to have some toxic action on the kidneys when inhaled as well as when ingested. Later experiments have shown the toxicity to be much lower (almost identical to the toxicity of ethyl alcohol) than they reported. However, kidney damage has been demonstrated when acetone is taken orally.[53]

Acetone is excreted rapidly, mainly by the lungs. In excessive exposures, some is also excreted through the skin and in the urine.[52]

Data on metabolism of acetone suggest that much of it is split to formate and acetate, to acetoacetate, and to the 3-carbon intermediates of glycolysis.[52]

3. Toxicity in Animals.

a. Single Oral Dose.

The toxicity of acetone in animals by this route is low. The lethal and narcotic doses in rabbits are reported to be 10 ml/kg and 7 ml/kg, respectively. In dogs, the lethal and narcotic doses were determined to be 8 ml/kg and 4 ml/kg, respectively.[52]

b. Single Intravenous Doses.

Lethal doses for rabbits and rats are 4 ml/kg and 6 to 8 ml/kg respectively. The narcotic dose was determined to be 2 ml/kg for both species.[52]

c. Single Intramuscular Doses.

Rabbits were depressed but not made unconcious when given 5 ml/kg intramuscularly.[52]

d. Skin Irritation.

Acetone may produce local dermatitis due to its defatting action on the skin if repeated prolonged contact occurs. An occasional short exposure should not cause skin irritation.[52]

e. Skin Absorption.

Lazarew *et al.*[54] reported that the amount of acetone absorbed through the skin was slight when they measured the amount exhaled and the amount in the blood following immersion of animal's foot.

f. Inhalation.

The inhalation effects of acetone are shown in table IV.[53] As with the other routes of administration, the inhalation toxicity of acetone in animals is low.

g. Eye Irritation.

Carpenter and Smyth[55] reported that small doses of acetone caused moderate irritation to the eyes of rabbits. Larson and co-workers[56] demonstrated mild edema. Gomer[57] suggested that dehydration of the sclera by the acetone resulted in gelatinous flocculation and opacity of the sclera. Injuries so incurred would be expected to resolve completely.

4. Effects in Man.

a. Repeated Oral Doses.

Albertone[58] reported that acetone taken orally in doses of 15 to 20 gm daily for several days produced no ill effects other than slight drowsiness.

b. Eye Effects.

Nelson and co-workers[59] reported that persons not accustomed to atmospheres containing acetone vapor experienced eye, nasal, and throat irritation when exposed to concentrations of 500 ppm. However, Oglesby *et al* (as cited in Patty[52]) found that acclimated persons could tolerate as much as 2500 ppm with only minor irritation of the nose and throat, that 200 to 400 ppm was detectable only upon immediate contact, and that after a short time, 700 ppm was undetectable.

c. Inhalation.

Patty[52] reported that Kagan, in experiments on himself, found that it was impossible to inhale acetone concentrations of 22 mg/ liter (9300 ppm) for longer than 5 minutes because of acute irritation of the throat. In addition to determining the intolerable concentration, Kagan also determined his absorption of inhaled vapor to be 71% for the 5-minute exposure. Two other men exposed by Kagan to 11 mg/l (4650 ppm) for 15 minutes absorbed 76 and 77% respectively.

Briggs and Schaffer, as cited by Patty[52], reported that the coefficient of distribution of acetone between alveolar air and blood or water was 1:333, expressed in mg/liter. Thus, a workman breathing 1000 ppm (2.3 mg/liter) of acetone in air would reach equilibrium when he had attained a blood concentration of 0.77 gm/liter. Under these exposure conditions, a man of average weight would accumulate 40 gm of acetone throughout the body. Once this level was attained, the only acetone absorbed would be that required to replace the amount metabolized or excreted and sufficient to equilibrate water consumed. Haggard, *et al.* also cited in Patty[52] demonstrated that this equilibrium is never actually reached even after several days of continuous exposure.

Table IV. Summary of Results of Inhalation of Acetone in Some Animals

Animal Species	Concentration		Duration of Exposure	Effects
	mg/liter	ppm	hr	
Mice	20	8,300	7-3/4	Side position after 4 to 7-3/4 hr; deep narcosis in only a few animals after 7-3/4 hr.
	48	20,000	1-1/2	Side position after 60-70 min; deep narcosis after 1-1/2 hr.
	110	46,000	1	Side position after 20-30 min; deep narcosis after 40-60 min; death a few minutes after end of experiment.
Cats	8–10	3,370–4,220	5	Initial salivation, irritation of nose and eyes; slight stupor and drowsiness after 5 hr.
	20–50	8,440–21,100	3–4	Usually drowsy in first 1/2 hr; later sleepy, increased sensitivity to pain.
	80–100	33,700–42,200	4	No drowsiness; marked irritation of central nervous system; giddiness, ataxia, narcosis, twitching and convulsions during narcosis.
	125	52,750	1-1/3	As above.
	40	17,000	4-1/2	Side position after 3-3/4 to 4 hr; recovery.
	114	48,000	3	Side position after 1-1/2 hr, no deep narcosis, recovery.
	178	75,000	1-1/2	Side position after 1/2-1hr; deep narcosis after 1 to 1-1/4 hr with preliminary convulsions.
Guinea pigs	50	21,000	25 min	Lacrimation only.
	50	21,000	4 to 8-1/2 9 to 23-1/2	Loss of auditory reflex; side position. Coma; death.

Patty cited Parmeggiani and Sassi[52] who showed that excretion of acetone in humans after a single oral dose is rapid for 8 hours, but is not complete in 24 hours. They reported that under conditions of light work and normal urination, the proportion of acetone excreted was approximately 40 to 70% in the breath, 15 to 30% in the urine, and 10% through the skin.

d. Skin Effects.

Acetone is only slightly irritating to the skin even after rather severe exposure. It may be slowly absorbed through the skin but this appears to be of little practical significance. The principal hazard to the skin involves the strong solvent action of acetone on skin lipids and other skin constituents. Damage of this kind only occurs with repeated, prolonged, and extensive skin contact.[52]

5. Summary.

The incidence of acute acetone poisoning is very low when the popularity of this solvent and the huge world-wide yearly consumption are considered. Although nonfatal industrial poisonings have been reported, they have resulted from the inhalation of high concentrations of acetone.[53]

Reports of poisonings due to repeated exposures have usually involved acetone as the solvent in combination with other materials. It is doubtful that the toxic symptons reported by workers who were affected could be attributed to acetone.[52]

Patty[52] cites the extensive studies conducted by Oglesby and co-workers over a 15-year period that represent 21 million man-hours of experience (average exposure concentrations up to 2000 ppm), and the 10-year studies performed by Fassett. Both groups have reported that no individual was injured by chronic acetone exposures. It is obvious from these data that the toxicity of acetone is low. The lethal dose for humans cannot be estimated.[52]

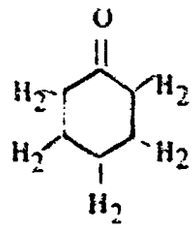
6. Hygienic Standards of Permissible Exposure.

The American Conference of Governmental Industrial Hygienists[60] has recommended a threshold limit of 1000 ppm (2400 mg/cu m) for acetone. This level is in good agreement with the results reported by Oglesby.[52]

E. Cyclohexanone.

1. Chemical and Physical Characteristics.

Cyclohexanone is a colorless, neutral liquid of peculiar ketone-like odor, with the following formula:



The molecular formula is $C_6H_{10}0$ and the molecular weight is 98.14. The boiling point is 155.6°C, the melting point is -45°C, the specific gravity 0.9478 20/4°C, and the vapor pressure 4.5 mm Hg at 25°C. Cyclohexanone is only slightly soluble in water but miscible with organic solvents. It is manufactured by catalytic oxidation of cyclohexanol and distillation of pimelic acid salts.[52,53]

Like many of the ketones, cyclohexanone is used both as a solvent and as a chemical intermediate. It has many applications in the lacquer, paint, and printing-ink trades. Its excellent solvent properties have made it desirable as a spot remover in the dry cleaning and textile industries. It is used in relustering and spray-painting fabrics. It is used as a degreaser, expecially in removing grease from nickel sheets. It is used in the leather industry as a thinner for fast-coating finishes on light and fancy leathers and for wet and dry degreasing in this trade. it improves the adhesion of varnishes, expecially on greasy leathers.[52,33]

It is stable and should not present a problem of flammability unless handled at elevated temperatures.[52]

2. Hazards of Physiological Action.

In the handling of cyclohexanone in industrial applications, skin and eye contact and inhalation of the vapors are most likely to occur. Ingestion or absorption of dangerous quantities through the skin are unlikely unless excessive exposures are encountered.[52]

Cyclohexanone is both an irritant and a narcotic agent. Death is thought to be due to respiratory failure. At high dosage levels, Treon *et al.*[61] found the organic sulfate and glucuronic acid output in the urine of rabbits increased.

No specific lesions were found in rabbits following exposure to lethal doses. Toxic effects were general vascular injury and inflammation. In repeated exposures of rabbits to smaller concentrations (190 ppm), liver or kidney changes were barely demonstrable and no significant blood changes were observed.[61]

Due to its low volatility, the possibility of obtaining hazardous levels during industrial handling is slight unless the process is conducted at elevated temperatures. The compound has strong warning properties (eye, nose, and throat irritation) at low concentrations; thus, overexposure to concentrations that may cause systemic injury are not likely to be tolerated voluntarily by most humans. Cyclohexanone has a low acute oral toxicity; occasional skin contact with the liquid should not be irritating but prolonged or frequent skin contact will cause defatting, irritation, or dermatitis. Eye contact may result in marked irritation and some transient corneal injury.[52]

3. Toxicity in Animals.

a. Single Oral Doses.

Jacobi, Hayashi, and Szubinski[62] reported the minimum lethal dose of cyclohexanone for mice to be 1.3 to 1.5 g/kg. Treated mice were seen to develop paresis of the hind quarters, narcosis, and deep, slow respiration before death. Treon, Crutchfield, and Kitzmiller[63] reported the LD100 value for rabbits to be 1.6 to 1.9 gm/kg and observed an increased excretion of organic sulfates and glucuronic acids in the urine. Some lung damage was seen at high dosages.

b. Single Intravenous Dose.

Patty[52] cited Caujolle and coworkers who reported that 630 mg/kg of cyclohexanone administered intravenously to anesthetized dogs caused death in 60 minutes. Accelerated respiration, vasodepression, and hypotension were noted.[52]

c. Single Intraperitoneal Injection.

Intraperitoneal injections of 0.5 ml/mouse were reported by Fillipe, as cited in Patty,[52] to cause excitation, paresis of hind quarters, marked hypothermia, and convulsions followed by death. One of the metabolic products found was adipic acid, presumably due to the oxidation of cyclohexanone.

d. Skin Irritation.

No published data are available on the effects of cyclohexanone on animal skin.

e. Skin Absorption.

Treon *et al.*[63] determined the LD100 by skin absorption in the rabbit to be 10.2 to 23.0 gm/kg. Tremors, narcosis, and hypothermia were reported prior to death. These effects are the same as those reported for other routes of administration but the dose required is larger.[52]

f. Eye Irritation.

Cyclohexanone applied to rabbit eyes caused marked irritation and some corneal damage. Therefore, liquid cyclohexanone may be expected to cause marked irritation and possibly some transient corneal injury when in contact with the human eye.[55]

g. Inhalation - Acute Exposures.

A 6-hour exposure of guinea pigs to 4000 ppm of cyclohexanone, as reported by Specht and coworkers,[64] caused typical narcotic symptoms: lacrimation, salivation, depression of body temperature and respiratory heart rates, and opacity of the cornea. Recovery from the narcosis was slow. Patty[52] cited Smyth, who found that a 4-hour exposure of rats to 8000 ppm resulted in anesthetic death but that a 4-hour exposure to 4000 ppm caused no deaths. When Gross (cited by Patty[52]) exposed mice, guinea pigs, and cats to 3800 ppm of cyclohexanone, the signs seen in the guinea pig by Specht[64] were noted. No abnormalities were found in the urine.

h. Inhalation - Repeated Exposures.

Patty[52] reports that monkeys and rabbits were exposed for fifty 6-hour periods to 190 ppm with no detectable effects other than very slight kidney and liver injury. At 309 ppm, slight eye irritation was seen; at 773 ppm, salivation and eye irritation were noted; and at 3082 ppm, the highest level used, light narcosis, labored breathing, incoordination, and a slightly increased mortality were seen. As in the single-dose oral studies, increased amounts of organic sulfate and glucuronic acid were found in the urine of the rabbits.[52]

4. Effects in Man.

a. Skin Effects.

Although no published data are available on the effects of cyclohexanone on human skin, it is reasonable to assume that frequent, repeated, or prolonged contact may possibly cause some irritation or dermatitis. This assumption is based on the fact that cyclohexanone is an excellent fat solvent and could dissolve the skin lipids and other constituents of the dermis. This hazard is considered to be of a low degree except for unusual situations where proper safety practices are not followed.[52]

b. Inhalation and Eye Effects.

Nelson and coworkers[59] exposed men to concentrations of 25, 50, and 75 ppm to determine the tolerable level for prolonged exposures. They reported 50 ppm was definitely objectionable, and that 75 ppm caused pronounced eye, throat, and nose irritation. A level of 25 ppm was thought by most volunteers to be the highest tolerable concentration for an 8-hour exposure.

5. Summary.

The principal hazard to health in handling cyclohexanone is inhalation of the vapors. However, because it is capable of defatting the skin, prolonged or frequently repeated skin contact may logically be expected to result in irritation or dermatitis.

6. Hygienic Standard of Permissible Exposure.

A threshold limit value of 50 ppm has been recommended by the American Conference of Governmental Hygienists.[60] This level should prevent narcosis but may be somewhat high, based on the work of Nelson *et al.*[59] If comfort is to be attained, the concentration of cyclohexanone in the air may have to be maintained below 50 ppm.[60]

III. EXPERIMENTATION, STUDIES OF THE TOXICITY OF RDX AND HMX IN DMSO, CYCLOHEXANONE, AND ACETONE CONDUCTED AT EDGEWOOD ARSENAL.

A. Intravenous Toxicity.

1. Mice.*

a. Procedures.

Ten percent (wt/vol) solutions of RDX in DMSO and HMX in DMSO were prepared at room temperature and injected into the caudal vein of 20-gm mice. The mice, in groups of six, received 5, 10, 15, 20, 25, or 30 mg/kg of RDX in DMSO, or 15, 25, 30, 35, or 50 mg/kg of HMX in DMSO. All mice were observed for 4 hr after injection and daily for 30 days.

* This part of the investigation was conducted by Edmund J. Owens, Toxicology Division and MAJ David G Fairchild, VC, Veterinary Medicine Division.

b. Results.

(1) RDX in DMSO.

The LD50 of RDX in DMSO administered intravenously to mice was calculated to be 18.71 (15.66-22.24) mg/kg from the data in Table V. Deaths occurred within 5 to 10 minutes and were preceded by mild convulsions and labored breathing. The survivors displayed lethargy which persisted for up to 2 hr; all appeared normal in 24 hr.

Table V. The Intravenous Toxicity of RDX in DMSO to Mice

Experimental		Bliss Analysis	
Dose	Mortality	Mortality	Dose
mg/kg		%	LD_{50} mg/kg
5	0/1	1	11.0 (5.9-20.4)
10	0/6	16	14.9 (10.7-20.8)
15	1/6	30	16.6 (13.0-21.2)
20	4/6	50	18.7 (15.7-22.3)
25	5/6	84	23.5 (18.4-30.1)
30	6/6	99	31.9 (18.9-53.8)
		Slope:	10.0

c. Discussion.

Solutions of DMSO containing either 10% HMX or RDX are of the same order of toxicity when administered intravenously to the mouse. It is estimated that between 10 and 20 ml of either solution would be required to present a lethal hazard to man if accidental injection occured.

2. Guinea Pigs *

a. Procedures

Solutions of RDX and HMX (each 33% wt/vol) in DMSO were prepared and given intravenously to guinea pigs, two animals per dose. Controls, two per dose, received 0.20, 0.23, and 0.25 ml of DMSO alone.

* Edmund J. Owens and David G. Fairchild conducted this investigation

b. Results.

Death occurred within 5 min after injection of either RDX or HMX solution. No signs were noted in the guinea pigs that received DMSO alone. The LD50, based on the data in table VI is 25.1 mg/kg of RDX and 28.2 mg/kg of HMX.

Table VI. Intravenous Toxicity of Solutions of RDX and HMX in DMSO in Guinea Pigs

Experimental			LD50 by Bliss analysis
Compound	Dose	Mortality fraction	(95% C.L.)
	mg/kg		mg/kg
RDX	15.8	0/2	25.1 (20.0 - 31.6)
	20.0	0/2	
	25.1	1/2	
	31.6	2/2	
HMX	20.0	0/2	28.2 (20.0 - 39.8)
	25.1	1/2	
	31.6	1/2	
	39.8	2/2	

B. Cutaneous Effects.

1. Rabbits.*

a. Procedures.

(1) Single Dose.

The backs of rabbits were clipped free of hair; those animals with observable skin abnormalities (abrasions, scratches, etc.) were not used. One milliliter of the following mixtures (wt/vol) were applied to six rabbits per mixture: 33% RDX or HMX in DMSO; 75% in cyclohexanone; and 5.4% in acetone. The HMX formed a suspension in cyclohexanone and acetone. Control animals, in groups of three, received 1-ml applications of the solvents alone. After all applications a polyethylene sleeve was taped to the back of each rabbit and left in place for 24 hr. When the sleeve was removed, each rabbit's back was examined for skin irritation and any irritation was graded according to FDA standards (table VII). The rabbits were observed daily for 30 days for evidence of skin irritation and systemic toxicity.

Blood samples were drawn from each rabbit and the following parameters were analyzed: red blood cell count, white blood cell count, hematocrit, hemoglobin, alkaline phosphatase, serum glutamic oxalacetic transaminase, blood urea nitrogen, creatinine, sodium, potassium, chloride, and carbon dioxide.

* This part of the study was performed by Ronald K. Biskup and Hubert L. Snodgrass, Toxicology Division.

Table VII. Gradations of Skin Effects (Draize Test)

Erythema	
No erythema	0
Mild erythema	1
Moderate erythema	2
Severe erythema	3
Erythema with edema	4
Necrosis	
No necrotic tissue	0
Less than 50% necrotic tissue	1
50% to 100% necrotic tissue	2
100% necrotic tissue with well-defined eschar formation	3
Dehydration and Desquamation	
No dehydration or desquamation	0
Mild dehydration or desquamation	1
Moderate dehydration or desquamation	2
Severe dehydration or desquamation	3

Two rabbits from each dose group and one control rabbit from each solvent group were sacrificed for pathological examination at 1 hr, 3 days, and 30 days.

(2) Repeated Doses.

The following solutions were prepared for repeated topical application to the clipped backs of rabbits: RDX – 33% in DMSO; 7.5% in cyclohexanone; and 5.4% in acetone; HMX – 33% in DMSO; 2.5% in cyclohexanone; and 2.0% in acetone. The compounds were applied in doses of 1.0 or 0.1 ml to six rabbits per mixture and volume; 5 days/week for 4 weeks. Control animals in groups of three received applications of 1.0 or 0.1 ml of the solvents alone. All rabbits were observed daily during the study for cutaneous or systemic effects. Skin irritation was graded as shown in table VII. The cumulative doses are listed in table VIII.

Blood samples were drawn from each rabbit and the same blood constituents analyzed as listed in the single dose study.

Two rabbits from each dose group and one control rabbit from each solvent group were sacrificed for pathological examination at 7 days (2 days after the fifth dose), 14 days (2 days after the 10th dose), and 28 days (2 days after the 20th dose).

(3) Supplemental Tests With HMX (33% in DMSO).

One-milliliter doses of HMX (33% in DMSO) were applied topically to the clipped backs of five sets of rabbits (two animals per set) daily for 1, 2, 3, 4, or 5 days. They were observed daily for 30 days for cutaneous and systemic effects.

Table VIII. Cumulative 1.0-Ml Doses Received by Rabbits in Repeated Topical Application Studies

Cumulative dose					
No. of doses	33% RDX or HMX/DMSO	7.5 RDX/Cyclohexanone	5.4% RDX/Acetone	2.5% HMX/Cyclohexanone	2.0% HMX/Acetone
			mg/kg		
1	165	37.5	27	12.5	10.0
2	330	75.0	54	25.0	20.0
3	495	112.5	81	37.5	30.0
4	660	150.0	108	50.0	40.0
5	825	187.5	135	62.5	50.0
10	1650	375.0	270	125.0	100.0
20	3300	750.0	540	250.0	200.0

b. Results.

(1) Single Dose.

Single doses of 1.0 ml RDX and HMX and the different solvents and the solvents alone produced no gross evidence of cutaneous irritation or systemic effects throughout the 30-day observation period. No changes in blood constituents that could be attributed to either the solvents or the dissolved explosives were noted. However, microscopic examination of the dosed areas showed that RDX in all solvents caused dermatitis that persisted for as long as 30 days. The solvent system causing the most pronounced effect was 33% RDX in DMSO. Dermatitis was not seen microscopically in any of the animals receiving doses of HMX solutions.

(2) Repeated Doses.

The repeated doses (daily 5 days/week for 4 weeks) of RDX in DMSO produced no gross evidence of cutaneous irritation throughout the 30-day observation period. Although no gross effects could be seen, a death occurred after the eighth application of the 1.0 ml dose of RDX in cyclohexanone (10th day of test), one death after the fifth application of the 0.1 ml dose of RDX in acetone (7th day), and another death after the 10th application of 1.0 ml dose of RDX in acetone (13th day).

Repeated doses (daily 5 days/week for 4 weeks) of 0.1 ml HMX in DMSO, 0.1 and 1.0 ml HMX in cyclohexanone and acetone produced no gross evidence of cutaneous irritation or systemic toxicity throughout the 30-day observation period. Of the solvents, only the 1.0-ml dose of crude DMSO had any effect, and this was a slight desquamation of the skin in the 2d week of application.

Repeated doses of 1.0 ml HMX in DMSO produced a mild desquamation of skin at 7 days. Three deaths occurred, one after the second application (2 days), one after the sixth application (8 days), and the other after the 20th application (30 days).

(3) Supplemental Tests With HMX (33% in DMSO).

Single and repeated doses of HMX in DMSO produced no evidence of cutaneous irritation. The two rabbits that received five applications appeared weak and dehydrated. Two deaths occurred after two applications (2d and 4th days), and one death after the fifth application (8th day). All survivors appeared normal upon gross examination 30 days after dosing.

(4) Pathological Findings.

Lesions which could be attributed to the compounds tested were confined to the site of application. When skin was affected, it was often reddened or thickened and there was microscopic evidence of inflammation. The incidence of dermatitis, as noted by the pathologists on necropsied animals, is shown in table IX. When minimal dermatitis occurred in animals that received the mixtures, there was dermatitis of a similar degree in the corresponding solvent control animal, with these exceptions. The animals treated with either 1, 10, or 20 1-ml doses of RDX in DMSO consistently had dermatitis at the time of necropsy while those receiving the same doses of DMSO alone did not. Two rabbits that received one 1.0-ml dose of RDX in acetone and two that received 20 0.1-ml doses of RDX in cyclohexanone had dermatitis and the solvent controls did not.

Table IX. Dermatitis* Produced in Rabbits Treated with Single and Repeated Topical Doses of RDX and HMX in DMSO, Acetone, and Cyclohexanone

Compound	Dose	Number of applications	Time of observation	Number of rabbits showing dermatitis					
				DMSO		Acetone		Cyclohexanone	
				Experimental	Control	Experimental	Control	Experimental	Control
	ml			33%		5.4%		7.5%	
RDX	1.0	1	1 hour	—	—	2/2	1/1	0/2	0/1
			3 days	2/2	1/1	2/2	0/2	2/2	1/1
			30 days	2/2	0/1	0/2	0/1	0/2	0/1
	0.1	5	7 days	0/2	0/1	0/2	0/1	0/2	0/1
		10	14 days	0/2	0/1	0/2	0/1	0/2	0/1
		20	30 days	0/2	0/1	0/1	0/1	2/2	0/1
	1.0	5	7 days	0/2	0/1	0/2	0/1	0/2	0/1
		10	14 days	2/2	0/1	0/2	0/1	0/2	0/1
		20	30 days	2/2	0/1	0/1	0/1	0/1	0/1
				33%		5.4%		7.5%	
HMX	1.0	1	1 hour	0/2	0/2	0/2	0/1	0/2	0/1
			3 days	0/2	0/1	0/2	0/1	0/2	0/1
			30 days	0/2	0/1	0/2	0/1	0/2	0/1
						2.0%		2.5%	
	0.1	5	7 days	0/2	0/1	2/2	0/1	2/2	0/1
		10	14 days	0/2	0/1	0/2	0/1	0/2	0/1
		20	30 days	0/2	0/1	0/2	0/1	0/2	0/1
	1.0	5	7 days	2/2	1/1	2/2	1/1	2/2	1/1
		10	14 days	0/2	0/1	1/1	1/1	2/2	1/1
		20	30 days	0/2	0/1	—	1/1	2/2	1/1

* Noted by pathologists on necropsied animals.

There was no difference in test and control animals receiving HMX in DMSO. Two rabbits that received five applications of 0.1 ml HMX in acetone and two that received five applications of 0.1 ml HMX in cyclohexanone had dermatitis while the controls were normal.

No lesions were found in the livers, kidneys, spleens, lungs, tracheas, hearts, intestines, bladders, muscles, bones, or bone marrows of the rabbits which died or were sacrificed following repeated topical applications of RDX or HMX in the three solvents, or the solvents alone. Gross examination of eyes revealed no cataracts. (Eye effects are discussed further in paragraph VI of this report.)

When presented for sacrifice and necropsy, three animals had signs of posterior leg weakness or posterior leg paralysis (possibly attributed to broken backs). They had been treated with ten 1.0-ml doses of HMX (2.5% in cyclohexanone), ten 1.0-ml doses of HMX (33% in DMSO), and five 1.0-ml doses of RDX (33% in DMSO), respectively.

c. Discussion.

The most serious hazard incident to handling the test solutions appears to be that of repeated skin contact with 33% HMX in DMSO (3 deaths), 5.4% RDX in acetone (2 deaths), and 7.5% in cyclonexanone (1 death). It is recommended, however, that workers avoid skin contact with any of the solvents to avoid damage to the human skin that might not be readily predicted from the response in rabbits.

2. Guinea Pigs.*

Solutions of RDX or HMX (33% wt/vol) were prepared and 316 to 1,000 mg/kg were applied to the clipped backs of guinea pigs in groups of four animals per dose. Observations are given in table X.

Table X. Effects of RDX and HMX Solutions in DMSO Applied to Backs of Guinea Pigs

Dose of explosive	Number of applications	Observations (4 animals)	
		RDX	HMX
mg/kg			
316	1	No effect	No effect
510	1	No effect	No effect
1,000	1	Slight erythema	Slight erythema
2,000	1	Slight erythema	Slight erythema
1,000	3	Slight erythema after first application; later applications showed no further erythema	Slight erythema after first application; later applications showed no further erythema. Skin spongy and absorbent after each application. After 3rd dose, apprehension, loss of weight, and loss of normal skin color were observed

Applications of 2 ml DMSO alone produced no effects.

* Ronald K. Biskup and Hubert L. Snodgrass, Toxicology Division, conducted this investigation.

C. Pharmacology of RDX and HMX in Unanesthetized Dogs.*

1. Experimental Procedures.

Forty-five healthy beagle dogs, averaging 11.4 kg, were used in a study to define the effects of RDX and HMX in various vehicles upon physiologic parameters, especially the central nervous system, of unanesthetized dogs after acute and chronic percutaneous application. In addition, a limited study of the toxic signs and the mechanism of action of these explosives after intravenous administration was conducted.

a. Surgical Preparation.

About 2 weeks before testing, electrodes were implanted under pentobarbital anesthesia into the skull of each dog above the cortical area for recording electroencephalograms (EEG). A longitudinal incision about 2-1/2 in. long was made across the scalp exposing the muscles, which were retracted over an area of the skull approximately 2 in. square. A limited amount of cauterization was used to retract the muscle and control bleeding. The exposed area of the skull was then scraped clean.

Two types of electrodes were used during the test series. The first consisted of a 5/8-inch-long nylon bolt with an 18-gage stainless steel core. The second was a round nylon plug (1/2 inch diameter) through which four silver-platinum wires were passed and extended underneath for about 1/4 to 1/2 inch.

To implant the first type of electrode, four holes were drilled through the skull to the dura. The holes were lateral to the midline, two being posterior to the external frontal crest and two in the central parietal area (figure A-1 in the appendix). The holes were then threaded and the electrodes were screwed in (two to three turns) and cemented in place with epoxy or acrylic. After the cement hardened, the skin and subcutaneous tissue were sutured back together and holes were cut into the skin so that the electrodes could be passed through.

To implant the second type of electrode, four small holes (0.04 in.), slightly larger than the diameter of the wire, were drilled into the skull in the same area as those for the first type of electrode. The wires and nylon plug were cemented in place and allowed to dry. The skin and subcutaneous tissue were pulled back firmly around the plug and sutured; the skin that bulged up around the electrode was excised.

The second type of electrode was found to be more durable for long term studies because the first type was more liable to be loosened when the dogs were in the holding cages. Both types recorded equally well.

Because of the length of the study (approximately 6 months), precautions had to be taken to prevent sickness or death from infection. Each animal was maintained on antibiotic therapy (Duracillin or Bicillin) for about 10 days after surgery and put back on therapy if any infection occurred during the holding time. The implant area was cleansed daily for 2 weeks with 3% hydrogen peroxide, and any necrotic tissue was swabbed away. In addition, some dogs had to be maintained on hydrocortisone injections for several days after the operative procedure to control inflamation and swelling.

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* This investigation was conducted by Henry P. Ciuchta, Ph. D., and SP4 J. Denny, Toxicology Division.

The administration of pentobarbital (Nembutal), 30 mg/kg_T iv, to dogs for surgical implantation or EEG electrodes produced typical changes in the wave pattern. Figure A-1 shows a representative tracing from a dog under the influence of the barbiturate compared to a tracing from the alert or unanesthetized animal (typical of all dogs prior to exposure).

b. Restraint.

Dogs used for the subacute percutaneous study were restrained in a stall-type holder consisting of two movable sheets of 1/4-in. pegboard (12 X 36 in.). Each animal was placed between two sheets and the pegboard was adjusted to fit snugly against the dog's flanks. Rods were then inserted through the holes to keep the dog in any desired position. Most of the dogs were relaxed and not bothered by this type of restraint.

c. Monitoring of Physiologic Parameters.

Blood pressure was measured by means of a catheter inserted into the femoral artery and attached to a Statham pressure transducer and carrier preamplifier. Electrocardiogram (EKG) and heart and respiratory rates were recorded from needle electrodes placed in either side of the chest wall, taped, and attached to an impedance pneumograph preamplifier and then to a cardiac preamplifier. The EEG was monitored by the implanted electrodes which were connected to a switch box and then to high gain preamplifiers. All the physiologic preamplifiers were then coupled to a six-channel E&M Physiograph Recorder or, in some instances, to a Sanborn 350 system. As depicted in figure A-1, a total of six EEG leads could be monitored, two at a time, by adjustment of the switch box. Leads I and II were used primarily for prolonged recording while Leads III through VI monitored other areas to determine whether similar activity was being shown at those points, i.e., if epileptiform discharges occurring in the frontal-parietal area were also occurring in the frontal or occipital areas, etc.

d. Tests Employed.

Dogs with implanted electrodes were given an ultrashort-acting barbiturate (Surital) on the morning of the experiment to permit placement of the catheter in the femoral artery. The animals recovered within 30 to 60 minutes and were then placed in the restrainers. Physiologic parameters (EEG, EKG, blood pressure, respiration, and heart rate) were monitored in order to establish control values. These same parameters were then assessed after a battery of 10 tests were presented to each animal to assure further that the animal's physiologic responses were normal prior to any exposure. These tests were as follows:

(1) Auditory. The normal responses of the animal to noise were assessed. The stimulus was generated by a bone audiophone (Sonotone Audiometer) placed near the ear of the dog with a frequency setting of 250 cps followed by 750 cps.

(2) Visual. A beam of light was passed in a vertical and then a horizontal line across the dog's field of vision.

(3) Pain. A pair of electrodes was placed against the inner aspect of a moistened ear and a Grass stimulator was set to discharge 25-volt shocks lasting 7.5 msec with a 2-msec interval between shocks. The stimuli were repeated until the animal's head jerked or the ear twitched.

(4) Eyelid. The eyelid was touched with a modified Von Frey hair (stainless steel 27-gage needle cleaner with a blunted end) until a blink occurred.

(5) Corneal. The center of the cornea was touched with the same wire until blinking occurred.

(6) Nasal. An ammonium hydroxide-impregnated cotton swab was passed under the nose for 1 to 2 seconds.

(7) Pupillary (Light). The size of the pupil was measured before and after a light (6-v, 5-amp bulb) was shone in the eye.

(8) Stroking. A metal bar was passed across the animal's skin on the back or neck, and neural response was noted.

(9) Light. A 6-v, 6-amp bulb was flashed for 1 second in front of the dog's eyes, and CNS response was noted.

(10) Vibratory (Rap). The restrainer holding the dog was rapped or hit with a metal object, and neural response was noted.

The responses to all these stimuli were recorded on the physiograph and are illustrated in figures A-2 to A-6 in Appendix A.

2. Exposures.

RDX and HMX were prepared in DMSO, acetone, and cyclohexanone but the concentrations were not all the same because of differences in solubility. The dogs were exposed either by topical application or intravenous injection (table XI).

The vehicles alone were administered topically in volumes of 1 ml and injected intravenously (0.125 ml/kg) into several dogs to assess activity.

The topical exposures were made by dropping 1 ml of RDX and HMX solutions onto the clipped backs of dogs. The animals were clipped weekly during the study.

Intravenous injections were given to two dogs through an indwelling catheter in the femoral vein. All other injections were made directly into the cephalic vein. Table XI shows how many dogs were exposed to each compound and to each vehicle alone.

The acute studies consisted of applying the test compounds to the dorsal area only once and recording blood pressure, respiratory and heart rates, EKG, and EEG at exposure time. The same parameters, except for blood pressure, were monitored weekly for the next 4 weeks. Blood pressure was recorded during the fourth week. The subacute studies were similar to the acute with the exception that the test compounds were applied daily 5 days per week for 4 weeks. Massive chronic exposures were an afterthought and entailed the percutaneous application of RDX and HMX in DMSO to two dogs each. The animals were exposed to 480 mg/kg daily for 3 consecutive days. The animals' EEG's, respiratory and heart rates, and EKG's were recorded at the time of each administration and at 1 week. The dogs were also observed grossly for any hyperreflexia. The intravenous studies were conducted primarily to determine potency and type of

Table XI. Exposure of Unanesthetized Dogs to RDX, HMX, and Solvents

Agent	Percutaneous						Intraveneous	
	Acute		Chronic[a]		Massive chronic[b]			
	Dose	No. of dogs	Dose	No. of dogs	Dose	No. of dogs	Dose	No. of dogs
	mg/kg		mg/kg		mg/kg		mg/kg	
33% RDX in DMSO	289.0	2	289.0	4	480.0	2	40.0 20.0	2 2
5.4% RDX in acetone	47.3	2	47.3	2			6.75 3.37	3 1
7.5% RDX in cyclohexanone	65.7	2	65.7	2			9.40 4.70 3.37	1 1 1
33% HMX in DMSO	289.0	1	289.0	4	480.0	2	40.0 20.0	6 2
2% HMX in acetone	17.5	1	17.5	2			2.5	3
5.4% HMX[c] in acetone							6.75	2
2.5% HMX in cyclohexanone	21.9	1	21.9	2			3.10 1.55	1 1
Solvents alone	ml		ml				ml (total dose)	
DMSO	1.0	1	1.0	2			1.0	4
Acetone	1.0	1	1.0	2			1.0	4
Cyclohexanone	1.0	1	1.0	2			1.0	3

[a] Applied daily 5 days per week for 4 weeks.

[b] Applied daily for 3 consecutive days.

[c] Not a clear solution.

pharmacologic activity. The dogs that were exposed by the iv route were observed subjectively for signs and symptoms such as twitching, convulsions, labored respiration, heart rate, salivation, lacrimation, cyanosis, prostration, and death.

If any of the animals in any phase exhibited subconvulsive jerking, twitching, etc., they were observed in the holding area after experimentation and were monitored periodically for hyperreflexia.

a. Topical Application.

(1) Acute.

Table B-I in appendix B shows the values obtained for blood pressure and heart and respiratory rates, before and after acute exposure, over the 4-week period. No consistent increase or decrease in any of the physiologic parameters was noted, and the high values for respiratory rate observed in some dogs reflected panting due to changes in the ambient temperature. Tables B-II and B-III depict trends in responses to test stimuli before and after acute exposure. The predominant responses were variations in respiratory and heart rates. No consistent enhancement or blockade of any of the responses monitored was noted. It also should be mentioned that all animals in this phase of the study showed a normal response to the lid, corneal, and visual tests before and after exposure. Responses to these tests were not tabulated.

(2) Subacute.

Table B-IV shows the values for blood pressure, heart rate, respiratory rate during the control-recording and the four subsequent weeks. No appreciable or consistent changes were observed at any time. Tables B-V and B-VI depict the trends in responses to the test stimuli before and after exposure. All animals in this phase also gave a normal response to the lid, corneal, and visual tests at all times. No blockade or enhancement of any physiologic response to a test stimulus was noted during the period. During the second or third week of application of DMSO alone or in combination with RDX and HMX some dogs exhibited slight erythema and desquamation of the back

(3) Massive Chronic.

The administration of 480 mg/kg of RDX and HMX in DMSO to four dogs (two with each test compound) for 3 consecutive days produced no consistent gross noticeable changes in the animals. Although one of the beagles receiving RDX-DMSO appeared to be slightly more irritable and hyperactive for 20 to 30 minutes after the application of the first and second doses, no disturbances were noted in the EEG. Animals were held for 2 weeks after exposure and appeared normal.

b. Intravenous.

To assess the type of activity that RDX, HMX, and the vehicles possessed once the compounds entered the circulation, a number of dogs were given the test compounds intravenously.

(1) Vehicle Controls.

Four experiments were conducted with 1-ml injections of DMSO. Two dogs showed no apparent changes in any parameters while two dogs demonstrated appreciable decreases in blood pressure (25 to 35 mm Hg), but only for 5 to 10 sec. Compensatory tachycardia occurred with the hypotension. Recovery was prompt (figure A-7). Four experiments conducted with 1-ml injections of acetone demonstrated decreases in blood pressure ranging from 10 to 50 mm Hg and being 5 to 60 sec in duration. Bradycardia, followed by tachycardia, was observed in 3/4 animals. A typical response to acetone is shown in figure A-8. The administration of 1 ml of cyclohexanone to three dogs produced a marked and immediate cardiovascular collapse with cardiac arrest lasting approximately 10 sec. Respiration was inhibited and pulse pressure became narrow during this phase. Figures A-9 to A-11 demonstrate these effects. Note the high-voltage, low-frequency activity of the EEG both at the 5-sec and 5-min intervals postinjection. Animals during this phase were comatose or semicomatose and had a dulled pain response. Recovery occurred 20 to 120 min after injection.

(2) RDX-DMSO (40 mg/kg).

Two dogs given this dose expired within 45 min and 90 min. Figure A-12 shows a typical response to RDX-DMSO at this dose. Within 15 to 30 sec animals demonstrated subconvulsive jerking, twitching, and convulsions. The seizures were cyclic and the latter phase of the experiment was marked by inadequate respiratory movements, decreased blood pressure, and a flat line on the EEG.

(3) RDX-DMSO (20 mg/kg).

Two dogs were given this dose. One animal demonstrated CNS hyperactivity within 15 sec after injection and exhibited hyperreflexia for at least 1 hr. Other parameters were not affected appreciably. The other dog convulsed within 90 sec after the injection and did not recover until 16 hr later (figure A-13).

(4) RDX-Acetone (3.37 and 6.78 mg/kg).

One dog was given the smaller dose and three the larger. The parameters monitored were generally unaffected except for decreased blood pressure and erratic EEG disturbances. All animals appeared normal after removal from the restrainer.

(5) RDX-Cyclohexanone (4.7 and 9.4 mg/kg).

One animal was tested with each dose and in both cases the effects were similar: marked decrease in blood pressure, cardiac arrest, and respiratory inhibition occurring in the presence of high-voltage, low-frequency EEG discharges. Animals were semicomatose to comatose, eyes were dilated and the pain threshold was elevated.

(6) HMX-DMSO (40 mg/kg).

Six dogs were given HMX-DMSO. Four were given a single dose of 40 mg/kg while two others received the same amount in two separate and equal doses. The single dose produced a severe cardiovascular collapse in all four animals. This occurred concurrently with a narrow pulse pressure,

bradycardia, and respiratory alterations (figure A-14). The EEG was characterized by high-voltage, low-frequency discharges. Two of the four animals died at 1 and 3 min, while two others survived the circulatory collapse. Vomiting was observed prior to their removal from the restrainer. Both expired in approximately 14± 2 hr.

Two animals receiving two separate doses of 20 mg/kg each exhibited a severe cardiovascular depression and one animal died in 1 min. The other dog recovered from the cardiovascular embarrassment but demonstrated EEG hyperactivity, vomiting, and extreme sensitivity to light and stroke stimuli at 1 to 2 hr after injection. The animal died in 14± 3 hr (figures A-15 to A-19).

(7) HMX-DMSO (20 mg/kg).

Two dogs were given a 20-mg/kg dose. In one animal (figures A-20 to A-22) there were minimal changes, although at 15 min the animal vomited. The dog then stabilized for 2 hr, at which time hyperreflexia to vibratory and light stimuli occurred. Visual perception and lid and corneal reflexes were normal. At 5-hr postinjection the dog became extremely hyperactive and had convulsive seizures. Recovery did not occur until 5 days after exposure.

In order to observe the toxic signs of HMX-DMSO without the influence of the restrainer, a 20 mg/kg dose of the compound was given to a second dog which was unrestrained and unoperated upon. The HMX-DMSO was injected in a volume of 0.6 ml into the cephalic vein of a 10-kg dog at 10:30 am. At 10:32 the animal became hyperpneic and retched. Defecation and salivation were observed at 10:37 am. At 10:47 am the dog became cyanotic; heart rate was 60 and respiratory rate was 120. At this point the animal was still standing although its pulse was becoming faint. At 10:53 am the dog was prostrate but capable of getting up. At 11:14 am the heart rate was 72 and respiratory rate was 120. For a short period of time the eyes reflected increased circulation and the pulse was getting stronger. At 11:20 am the dog was cyanotic again. Retching occurred at 11:56 am and again at 12:22 pm. At 1:40 pm breathing became labored. At 1:41 pm there was an onset of subconvulsive jerking, especially in response to the auditory stimulus. Clonic-tonic convulsions occurred at 1:47 pm and again at 2:01 pm. Opisthotonus was noted at 2:02 pm. At 3:00 pm the animal was convulsing. The dog sat up at 3:20 pm but hyperreflexia was pronounced. Convulsions occurred again at 4:07 pm. The animal was prostrate at 4:45 pm and died between 10:00 pm and 3:00 am.

(8) HMX-Acetone (2.5 mg/kg of 2% and 6.75 mg/kg of 5.4%).

The administration of the lesser dose to a dog produced transient hypotension. The EEG demonstrated a sleep-like wave pattern during the 12- to 40-min postinjection period, but the animal could be aroused easily. No irregularities were noted the following day. The larger dose produced a blood pressure decrease along with high-voltage, low-frequency EEG discharges. Upon removal from the restrainer the animals were lethargic, but all appeared normal the next day.

One dog was given 6.75 mg/kg (5.4%) HMX-acetone after receiving a prior injection of 2.5 mg/kg of the 2% solution. The second dose produced a drop in blood pressure, cardiac arrest, and a depressed respiratory rate. The EEG was not adversely affected. At various intervals the dog vomited and periodically fell asleep but could be aroused (figures A-23 to A-27). The animal appeared normal the next day.

(9) HMX-Cyclohexanone (1.55 and 3.1 mg/kg).

One dog was used for each dose. Both demonstrated cardiovascular collapse, onset of a comalike state, and elevation of the pain threshold. The animal receiving the larger dose retched, had tremors, and vomited. No convulsive seizures were noted. Both dogs recovered completely in 2 to 3 days.

3. Discussion.

The absence of consistent changes in blood pressure, heart rate, respiratory rate, EKG, and EEG over a 4-week period after topical application of RDX and HMX in the vehicle indicates that the mixtures do not penetrate the skin at the doses and concentrations tested in the acute and subacute experiments. This is supported by the fact that intravenous administration of these materials causes changes in the physiologic parameters.

One of the two dogs receiving the massive dose of RDX-DMSO appeared to demonstrate slight hyperexcitability approximately 15 to 30 min after the second and third application of the compound. Since these particular animals were held for so long, they even appeared anxious in the control phase and a more involved study would have to be conducted for substantial evaluation.

The majority of the intravenous studies were conducted with RDX and HMX in DMSO. Although iv injections of the agents in acetone and cyclohexanone were also evaluated, it was found that these vehicles themselves (figures A-8 to A-11) exerted a depressant action on the cardiovascular system, and cyclohexanone induced changes in the EEG pattern. Animals exposed to cyclohexanone also demonstrated a semicomatose to comatose condition in some instances. RDX-acetone did cause some EEG disturbances, but these were not well defined. Further difficulty was encountered because the concentrations of the active agents in these vehicles was considerably less in DMSO. DMSO itself was shown (figure A-7) to produce relatively little effect when administered intravenously, and no gross complications were observed during a holding period of 2 weeks.

It is evident from these studies that the immediate effects of RDX and HMX differ, RDX affecting the CNS immediately after injection and HMX producing a circulatory collapse initially, with delayed CNS disturbances.

Our RDX studies seem to bear out Sunderman's work[66] which suggested that RDX itself and not a breakdown product was responsible for the CNS effects. He demonstrated a rapid onset of symptoms after intraperitoneal and iv injections, and we experienced similar results with iv administration. Von Oettingen[3] had previously considered that an amine may be degraded and cause CNS disturbances because it is known that certain aliphatic amines may cause a toxicologic response similar to those that he observed with continued RDX feedings.

Von Oettingen's studies have also indicated that oral feedings of RDX to dogs (10% acacia mixture) produced questionable, or no physiological effects when the dose ingested was 5 to 15 mg. However, those dogs were pretreated with amytal and undoubtedly the barbiturate influences the response to any compound that may have a CNS effect. In addition, the dose buildup in the stomach was not comparable to our iv dose. Sunderman[66] has shown that CNS disturbances

can be abolished by decerebration or administration of Nembutal, thereby indicating a CNS involvement at a higher level.

RDX feedings, according to Von Oettingen[3] and Sunderman,[66] produced no pathologic changes in the brain, although nonspecific lesions were observed in the renal tubules, the liver, and the heart muscle. Slanskaya and Pozharsky,[5] however, observed pathologic changes in various organs as well as the CNS after acute and chronic feedings. In acute cases the CNS vascular supply appeared to be impaired because of changes in the vessel walls. Nerve cell degeneration was also observed. The areas most affected in descending order were: spinal cord, brain stem, and cortex.

From the pharmacologic activity of HMX in these limited iv studies, one could assume that this agent is more of a "nitritelike" compound, unlike RDX. Nitrites, however, are known to produce methemoglobinemia, which may induce respiratory embarrassment and, in conjunction with a collapsed circulation, may bring about convulsive seizures. However, we did not observe the typical chocolate-colored blood indicative of methemoglobin in a small sampling of dogs.

At this point several possibilities might explain HMX activity:

1. HMX produces a nitritelike effect on the cardiovascular system; if respiration and circulation are severly impaired and methemoglobin is formed, CNS disturbances result;

2. Because of some inherent chemical property, it takes longer for HMX to exert CNS effects than it does for RDX;

3. HMX produces a circulatory effect initially, followed by recovery and then CNS effects because of some specific action of the agent which may be dependent upon some time-consuming metabolic pathway.

One has to bear in mind that CNS disturbances were still apparent in some of our HMX-poisoned dogs even after their blood pressure and respiration recovered to what one would think were adequate levels.

In order to be more definitive in the evaluation of HMX, pharmacologic activity studies should be conducted in enough animals to assess the cardiovascular, respiratory, and hematologic pictures during various stages of response.

4. Conclusions

There was no evidence that either explosive agent (RDX or HMX) had any physiologic effect upon the dog when acute and subacute exposures were made topically. The intravenous administration of RDX, depending on the dose administered, produced CNS hyperactivity, tremors, convulsions, and death. HMX, on the other hand, initially produced cardiovascular collapse, which in some cases led to immediate death or, depending on the dose, to recovery and eventually to CNS disturbances.

D. Sensitization Potential of RDX and HMX.*

1. Procedures.

Sensitization studies of RDX and HMX in acetone, cyclohexanone, and DMSO were conducted using the experimental methods of Landsteiner[67-71] and others who followed his classic procedures. Sensitization effects were evaluated using the Draize test as recommended by the US Department of Agriculture, Food and Drug Administration (table VII, page 27). Young adult guinea pigs of both sexes were exposed to the test compounds by topical application to the skin and by intradermal injection.

For each topical application, 0.5 ml of test compound was applied. Acetone and cyclohexanone solutions were applied as free-falling drops dispensed (through a 20-gage needle) from a 2.5-ml hypodermic syringe. DMSO, pure and technical grade, was dispensed in the same way but it was necessary to spread it out with a sterile, cotton-tipped applicator because the material was quite viscous and tended to bead on the skin surface. All animals were clipped several hours before exposure to encourage maximal skin penetration. The skin was not covered after exposure in order to simulate the conditions of a "spill exposure."

For intradermal injection, 0.05-ml volumes of test solution were given with a 0.5-ml hypodermic syringe attached to a 26-gage needle. Each exposure site was clipped several hours before administration and sterilized with ethyl alcohol immediately before injection.

These sensitization studies were divided into three areas: the senzitizing phase; the rest phase; and the challenge phase.

During the sensitizing phase the guinea pigs were exposed to the test compounds 3 days per week for 3 weeks. Either topical or intradermal doses were applied to the clipped dorsal thorax and observations for skin effects at the site of the exposure as well as for systemic toxicity were made daily.

During the rest phase (2 weeks) no compounds were administered and the animals were observed daily. Concurrent with this phase, experiments were conducted to determine the maximum suberythemal dose to use during the challenge phase. Serial dilutions of the sensitizing stock solution were made in saline or polyethylene glycol (PEG 200) and injected into a group of naive guinea pigs until the dose was found that caused no skin effects. In this study of RDX and HMX, considerable time and effort were devoted to obtaining this subeffective dose because of the activity of the solvent used.

In the challenge phase, the clipped thighs of the animals were exposed intradermally and topically to single doses of test material at the predetermined maximum subeffective level. Although one route per animal was used in the sensitization phase, either intradermal or topical, both routes were used to challenge, one on each thigh at different times. The animals were observed daily for 3 days after dosing for signs of skin effects at the application site. The challenge doses of RDX and HMX were prepared in 1:1 (v/v) solvent-saline mixtures (intradermal) and PEG (topical). Using the nonirritating PEG eliminated the need for excessive dilution allowing substantial amounts

* John F. Callahan, Toxicology Division, performed the sensitization investigation.

of the compounds to be applied. The erythematous effects of undiluted cyclohexanone and DMSO were also eliminated and the contact persistency of the challenge material was increased. Some animals were also exposed to diluted (intradermal) and undiluted (percutaneous) doses of the three solvents alone and then challenged.

2. Results.

Initial experiments for sensitization with topical HMX in DMSO were conducted using 0.5 ml of 33% HMX/DMSO solutions. Deaths occurred after the first and second sensitization exposure (5/12 animals). Two animals showed signs of hyperirritability and intermittent convulsions within 24 to 48 hours after one or two exposures. One of these animals died within 24 to 48 hours after the first treatment; the other survived and received all of the scheduled exposures. The other deaths occurred overnight and toxic signs were not observed. These deaths were apparently due to HMX poisoning since other animals receiving DMSO alone showed no toxic signs and did not die.

When the topical sensitizing doses were reduced to the following, none of the animals died during the sensitizing period:

2% HMX in acetone

2.5% HMX in cyclohexanone

3.3% HMX in pure and technical grade DMSO

100% acetone, cyclohexanone, and DMSO

5.4% RDX in acetone

7.5% RDX in cyclohexanone

33% RDX in pure and technical grade DMSO

Because the solvents used are known skin irritants, the animals were carefully observed throughout the sensitization period for skin effects. The differences in skin effects between RDX- and HMX-solvent mixtures and the effects from the solvents alone were evaluated. The results are shown in table XII and XIII.

It was readily seen that acetone, with or without RDX or HMX, cause no skin effects. This probably is related to its high volatility. Significant skin effects occurred in animals receiving repeated topical exposure to cyclohexanone or DMSO, with and without RDX or HMX. Most animals had significant skin effects from DMSO by the end of the first week. Similar effects were not seen in most animals until the end of the second week of application of cyclohexanone. The intensity of effects of both solvents were generally increased by the end of the third week. Although not shown in the tables, these effects, with or without RDX and HMX, diminish when treatment is stopped. Visual inspection of the exposure sites showed absence of effects 18 to 24 days after the final treatment.

No significant differences between the skin effects caused by the two solvents alone and those caused by RDX- and HMX-mixtures were evident. It is apparent, therefore, that DMSO

Table XII. Residual Skin Effects Caused by the Repeated Topical Application of 0.5 ml RDX and/or Several Solvents on the Backs of Clipped Guinea Pigs (Sensitization Period)

Fraction of group showing residual topical skin effects[a]

Treatment	Cumulative number of treatments[b]								
	1	2	3	4	5	6	7	8	9
27 Mg of RDX in acetone (5.4% RDX)	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
37.5 Mg of RDX in cyclohexanone (7.5% RDX)	0/6	0/6	0/6	1/6 s	1/6 s 1/6 S	1/6 se 2/6 S 1/6 Sc	2/6 s 2/6 s 4/6 Sc	1/6 s 4/6 S 1/6 Sc	5/6 S 1/6 Sc
165 Mg of RDX in pure DMSO (33.0% RDX)	0/6	0/6	1/6 s 4/6 S	3/6 S 2/6 S	2/6 S 4/6 S	1/6 s 4/6 S 1/6 Sc	4/6 S 1/6 Se 1/6 Sc	1/6 s 3/6 S 1/6 S 1/6 Se	1/6 s 2/6 S 3/6 S
165 Mg of RDX in tech grade DMSO (33.0% RDX)	3/6 S	3/6 S	2/6 S 3/6 S	3/6 S 1/6 Se 2/6 Sc	1/6 S 1/6 Se 1/6 S 2/6 Sc	1/6 Se 1/6 S 4/6 Sc	1/6 Se 1/6 S 4/6 Sc	2/6 S 4/6 Sc	2/6 S 4/6 S
Acetone	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Cyclohexanone	0/6	0/6	0/6	1/6 e	1/6 e	4/6 s 1/6 se	3/6 S 1/6 Se 1/6 Sc	4/6 S 1/6 S	2/6 S 4/6 Sc
Pure grade DMSO	3/6 S	6/6 S	2/6 S 3/6 S	2/6 S 2/6 Se 1/6 SE	3/6 S 2/6 Se	1/6 s 2/6 S 2/6 Se 1/6 Sc	4/6 S 1/6 Se 1/6 Sc	1/6 s 1/6 Se 1/6 Sc	1/6 s 4/6 S 1/6 S
Tech grade DMSO	1/6 S	4/6 S	6/6 S	3/6 S 3/6 Se	3/6 S 2/6 Se 1/6 S	1/6 S 2/6 Se 1/6 Se 1/6 Sc	1/6 S 2/6 S 1/6 Se 1/6 Se 1/6 Sc	1/6 S 4/6 S 1/6 Sc	2/6 s 4/6 S

a/ Based on observations made 48 to 72 hr after each treatment.

b/ Percutaneous treatments were given 3 times per week over a 19-day period.

Cyclohexanone and both types of DMSO, with and without RDX, had immediate effects after a single application. Mild erythema was observed in all animals exposed to cyclohexanone and lasted from 1 to 6 hrs. Both types of DMSO induced a moderate erythema which subsided to a mild erythema by 6 hr and was usually absent at 24 hr.

LEGEND: s: Slight scaling of the skin.
S: Moderate scaling of the skin.
S: Heavy scaling of skin.
Sc: Moderate scaling of skin with mild erythema.
SE: Moderate scaling of skin with moderate erythema.
Sc: Moderate scaling of skin with cracking.
Sc: Heavy scaling of skin with cracking.

Table XIII. Residual Skin Effects Caused by the Topical Application of 0.5 ml HMX and/or Several Solvents on the Backs of Clipped Guinea Pigs (Sensitization Period)

Fraction of group showing residual topical skin effects[a]

Treatment	Cumulative number of treatments[b]								
	1	2	3	4	5	6	7	8	9
10 Mg HMX in acetone (2% HMX)	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
12.5 Mg HMX in cyclohexanone (2.5% HMX)	0/6	0/6	0/6	0/6	0/6	4/6 S	6/6 S	5/6 S 1/6 Se	2/6 S 4/6 Sc
16.5 Mg HMX in pure grade DMSO (3.3% HMX)	0/6	3/6 S	2/6 S	5/6 S 1/6 Se	6/6 S	5/6 S	3/6 S 2/6 Sc	2/6 S 2/6 SC 1/6 Sc	2/6 s 2/6 S
165 Mg HMX in pure grade DMSO (33% HMX)	0/5[c]	0/4	0/4	1/4 S	3/4 S	4/4 S	4/4 S	1/4 S 3/4 Sc	3/4 S
16.5 Mg HMX in tech grade DMSO (3.3% HMX)	0/6	2/6 S	1/6 S	6/6 S	6/6 S	6/6 S	5/6 S 1/6 Sc	5/6 S 1/6 Sc	3/6 s
165 Mg HMX in tech grade DMSO (33% HMX)	0/3[d]	0/3	0/3	2/3 S	2/3 S	2/3 S	3/3 S	3/3 S	3/3 s
Acetone	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Cyclohexanone	0/6	0/6	0/6	0/6	0/6	5/6 S	6/6 S	6/6 S	2/6 S 4/6 Sc
Pure grade DMSO	0/6	0/6	1/6 S	2/6 S	3/6 S	6/6 S	5/6 S	5/6 S 1/6 Sc	2/6 s 3/6 S
Tech grade DMSO	0/6	0/6	1/6 S	3/6 S	5/6 S	5/6 S	6/6 S	5/6 S 1/6 Sc	3/6 s 2/6 S

[a] Based on observations made 48 to 72 hr after the second treatment. Cyclohexanone and both types of DMSO, with and without HMX, had immediate effects after a single application. Mild erythema was observed in all animals exposed to cyclohexanone and lasted from 1 to 6 hr. Both types of DMSO induced a moderate erythema which subsided to a mild erythema by 6 hr and was usually absent at 24 hr.

[b] Percutaneous treatments were given 3 times per week over a 19-day period.

[c] One animal died within 24 to 48 hr after the first treatment and a second animal died within 24 to 48 hr after the second treatment.

[d] Three animals died within 24 to 48 hr after the first treatment.

LEGEND: s: Slight scaling of the skin.
S: Moderate scaling of the skin.
S̲: Heavy scaling of the skin.
Se: Moderate scaling of the skin with mild erythema.
SE: Moderate scaling of the skin with moderate erythema.
Sc: Moderate scaling of the skin with cracking.
S̲c̲ : Heavy scaling of the skin with cracking.

and cyclohexanone produce skin irritation when administered topically and that RDX and HMX make little or no contribution.

Similar evaluations were made of the effects caused by RDX- and HMX-solvent mixtures and the solvents alone when these materials were administered intradermally (table XIV). As would be expected, the severity of skin effects was much greater after intradermal injection than after topical application. All solvents caused moderate to severe erythema, edema, and necrosis during the course of repeated intradermal exposures. Just as in the topical exposures, RDX and HMX apparently contributed little to these effects. The results shown in table XIV indicate that cyclohexanone caused the most severe effects, acetone the intermediate, and DMSO the least severe effects. All three solvents are considered unsafe for intradermal injection at the concentrations used in this study.

Tables XV and XVI show the work done to determine suberythemal doses for topical and intradermal challenges with RDX-solvent mixtures. Tables XVII and XVIII show similar determinations for HMX-solvent mixtures by both routes.

The suberythemal doses for RDX in acetone and in DMSO for intradermal challenge were 1:32 and 1:16, respectively, saline dilutions of the stock solution used in the sensitization phase. For the RDX-cyclohexanone mixture, it was a 1:64 dilution. The intradermal suberythemal dose of HMX in acetone and DMSO was 1:16 and 1:32, respectively, saline dilution and for cyclohexanone, a 1:64 saline dilution. For reasons previously explained, the diluent used for determination of a suberythemal dose for percutaneous challenge was PEG 200. The suberythemal dose for both HMX and RDX was found to be a 1:10 dilution of the stock solution.

As stated in the procedure, 10 distinct sensitization potential studies were completed with challenges by the suberythemal dose; four with RDX-solvent mixtures, four with HMX-solvent mixtures, and two with the solvents alone. The various combinations are listed below along with the tables in the appendix that show the results.

	Routes of Administration		
Compound	Sensitization	Challenge	Table no.
RDX	Intradermal	Topical	B-VII
	Intradermal	Intradermal	B-VIII
	Topical	Topical	B-IX
	Topical	Intradermal	B-X
HMX	Intradermal	Topical	B-XI
	Intradermal	Intradermal	B-XII
	Topical	Topical	B-XIII
	Topical	Intradermal	B-XIV
Solvents	Intradermal	Topical	B-XV
	Intradermal	Intradermal	B-XVI

No evidence of sensitization was found from any of the combinations of exposures to RDX-solvents, HMX-solvents, or the solvents alone.

Table XIV. The Skin Effects of 0.05 ml of 1:1 Saline Mixtures of Acetone, Cyclohexanone, and Pure and Technical Grade DMSO, With and Without RDX and HMX, Applied Intradermally to the Clipped Dorsal Thorax of Guinea Pigs

Treatment	Time Post Treatment (Days)											
	1	2	4	7	9	11	14	16	18	21	23	28
19.8 mg of acetone	2-EdB 1-edB 1-eB	2-EdB 1-edB 1-eB	4-EdB	4-EdN	4-EdN	4-EdN	2-EDN 1-EdN 1-edN	-*	2-EdN 1-eNS 1-eS	-		2-eS 1-enS 1-NS
0.125 mg RDX in acetone (0.25% RDX)		SEd 1-e	3-Ed 1-ed 2-e	1-ED 1-ED 1-Ed 1-ed 2-e	3-EDN 1-ed 2-e	1-EDN 1-EDN 1-EdN 1-ed 1-e 1-O	1-EdN 4-e 1-0	5-eS 1-S	5-eS 1-S	5-eS 1-S	6-S	6-S
0.125 mg HMX in acetone (0.25% HMX)	-	3-EdN 3-Ed	2-EDN 1-EdB 1-edB 1-EDN 1-EdN	5-EDN 1-EdN	5-EDN 1-EdN	5-EDN 1-EDN	6-EDN	1-EDN 5-EnS	2-eS 4-enS	4-eS 2-enS	4-eS 2-enS	6-eS
23.4 mg of cyclohexanone	2-EdB 1-EB 1-edB	3-EdB 1-eB	3-EdN 1-EdN	3-EdN 1-EdN	4-EdN	4-EdN	4-EdN	-	3-EDN 1-eS	-		1-enS 1-nS 2-S
0.125 mg RDX in cyclohexanone (0.25% RDX)	-	1-ED 2-Ed 2-eD 1-ed	1-ED 3-ED 2-Ed	6-ED	1-EDN 2-EDN 3-EDN	2-EDN 1-EdN 2-EDN 1-Ed	1-ed 1-edN 4-e	1-ed 5-eS	1-ed 5-eS	4-eS 2-S	6-S	6-S
0.125 mg HMX in cyclohexanone (0.25% HMX)	-	6-EdN	3-EDN 3-EdN	6-EDN	3-EDN 1-EDN 2-EDN	4-EDN 1-EDN 2-EDN	5-EDN 1-EdN	3-EDN 1-EnS 2-enS	1-EDN 1-EdN 2-eS 1-S	1-EdN 1-edN 4-eS	2-edN 1-enS 3-eS	6-eS
27 mg of pure DMSO	1-EdB 1-edB 1-ed 1-eB	1-EdB 1-edB 1-ed 1-eB	2-EdB 1-edB 1-edN	1-EdB 1-edB 2-edN	1-EdB 3-edN	1-EdB 3-edN	1-edN 2-en 1-S	-	3-eS 1-nS	-		6-S
0.125 mg HMX in pure DMSO (0.25% HMX)		4-Ed 1-ed 1-e	1-EdB 3-Ed 2-e	1-EdN 1-Ed 3-edN 1-e	1-EdN 5-edN	2-EdN 4-edN	1-edN 1-enS 3-eS 1-S	1-enS 3-eS 2-S	4-eS 2-S	1-enS 2-eS 3-S	1-enS 3-eS 2-S	6-S
27.4 mg of tech grade DMSO	1-EdB 3-edB	1-EdB 1-edN 1-eB 1-e	2-edB 1-edN 1-e	4-edN	4-edN	4-edN	4-en	-	1-enS 2-nS 1-eS	-		6-S
0.125 mg RDX in tech grade DMSO (0.25% RDX)	-	1-E 2-Ed 2-ed 1-e	2-Ed 2-ed 2-e	2-EdN 2-Ed 1-ed 1-e	2-edN 3-ed 1-e	2-edN 3-ed 3-e	3-eS 3-e	6-eS	6-S	6-S	6-S	6-S
0.125 mg HMX in tech grade DMSO (0.25% HMX)		5-EdB 1-EdN	5-EdN 1-edN	5-EdN 1-edN	6-EdN	5-edN 1-eS	5-enS 1-eS	5-eS 1-S	6-eS	5-eS 1-S	5-eS 1-S	6-S

* Not observed on that day.

LEGEND: O: No effect
E: Severe erythema
E: Moderate erythema
e: Mild erythema
D: Severe edema
D: Moderate edema
d: Mild edema
N: Severe necrosis
N: Moderate necrosis
n: Mild necrosis
B: Blanched tissue
S: Scar tissue formation

Table XV. The Determination of a Suberythemal Dose of Intradermally Administered RDX in Acetone, Cyclohexanone, and Technical Grade DMSO in Clipped Guinea Pigs

Dilution of stock	Skin effects[a] RDX:Acetone[b] No. of Animals	Observation time 24 hr	48 hr	72 hr	RDX:Cyclohexanone[b] No. of animals	Observation time 24 hr	48 hr	72 hr	RDX:DMSO (Tech Grade)[b] No. of animals	Observation time 24 hr	48 hr	72 hr
Stock	2	2-E4	2-E4	2-E4	2	2-E4	2-E4	2-E4	4	4-E4	4-E4	4-E4
		1-N2	1-N2	1-N2		2-N2	2-N2	2-N2			1-N2	1-N2
1:2	4	4-E4	4-E4	4-E4	2	2-E4	2-E4	2-E4	4	2-E4	2-E1	0
						2-N2	2-N2	2-N2		1-E1		
1:4	4	4-E1	4-E1	1-E1	2	2-E4	2-E4	2-E4	4	2-E1	2-E1	1-E4
						1-N2	1-N2	2-N2				
1:8	4	1-E1	0	1-E1	2	2-E4	2-E4	2-E4	4	2-E1	2-E1	1-E1
1:16	4	2-E1	0	0	4	4-E4	2-E4	2-E4	2	0	0	0
							1-E1					
1:32	4	0	0	0	4	4-E1	2-E1	2-E1	2	0	0	0
1:64	4	0	0	0	2	0	0	0	2	0	0	0

[a] The Draize Test was used to evaluate skin effects (table VII, p. 27).

[b] 0.05 ml of 0.25% RDX in 1:1 solvent: saline solution.

Table XVI. The Determination of a Suberythemal Dose of RDX in Acetone, Cyclohexanone, Pure and Technical Grade DMSO When Administered Topically in PEG 200 in Clipped Guinea Pigs

Compound	Solvent	Dilution in PEG 200	No. of animals exposed[a]	Skin effect[b] 4-5 hr	24 hr	48 hr	72 hr
PEG 200 (100%)	–	–	16	0	0	0	0
RDX (5.4%)	Acetone	1:10	3	0	0	0	0
RDX (5.4%))	Acetone	1:100	3	0	0	0	0
RDX (7.5%)	Cyclohexanone	1:10	3	0	0	0	0
RDX (7.5%)	Cyclohexanone	1:100	3	0	0	0	0
RDX (33.0%)	Pure DMSO	1:10	3	0	0	0	0
RDX (33.0%)	Pure DMSO	1:100	3	0	0	0	0
RDX (33.0%)	Tech grade DMSO	1:10	3	0	0	0	0
RDX (33.0%)	Tech grade DMSO	1:100	3	0	0	0	0

[a] 0.5 ml

[b] The Draize TEst was used to evaluate skin effects (table VII, p. 27).

Table XVII. The Determination of a Suberythemal Dose of Intradermally Administered HMX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO in Clipped Guinea Pigs

Dilution of Stock	Skin effects[a]																			
	0.25% HMX: acetone[b]					0.25% HMX: cyclohexanone[b]					0.25% HMX: pure DMSO[b]					0.25% HMX: DMSO (tech grade)[b]				
	No. of animals	Observation time				No. of animals	Observation time				No. of animals	Observation time				No. of animals	Observation time			
		2-3 hr	24 hr	48 hr	72 hr		2-3 hr	24 hr	48 hr	72 hr		2-3 hr	24 hr	48 hr	72 hr		2-3 hr	24 hr	48 hr	72 hr
1:2	2	2-E4	2-E4	2-E4	2-E4 1-N2		–	–	–	–		–	–	–	–		–	–	–	–
1:4	4	1-E1	2-E1	1-E1	0	2	1-E4 1-I1	1-E4 1-N2 1-E1	1-E4 1-N2 1-E1	1-E4 1-N2 1-E1	2	0	1-E4 1-E1	1-E4 1-E1	1-E1 1-E1	2	0	1-E1	0	0
1:8	4	1-E1	2-E1	0	0	2	1-E4 1-E1	2-E4 2-N2	2-E4 2-N2	2-E4 2-N2	4	1-E4	1-E1	1-E1	0	4	1-E4	1-E4	1-E4	1-E
1:16	2	0	0	0	0	4	1-E1	2-E4 1-E1	2-E4 1-E1	2-E4	4	0	1-E1	0	0	4	0	0	1-E1	0
1:32	4	0	0	0	0	4	0	1-E1	0	0	6	0	0	0	0	6	0	0	0	0
1:64	5	0	0	0	0	7	0	0	0	0	5	0	0	0	0	4	0	0	0	0
1:128	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0

[a] The Draize Test was used to evaluate skin effects (table VII, p27).

[b] 0.05 ml

Table XVIII. The Determination of a Suberythemal Dose of HMX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO When Administered Topically in Polyethelene Glycol 200 in Clipped Guinea Pigs.

Compound	Solvent	Dilution in PEG 200	No. of animals exposed[a/]	Skin effects[b/]			
				3 hr	24 hr	48 hr	72 hr
				No. animals/effects			
PEG 200 (100%)	–	–	16	0	0	0	0
HMX (2.0%)	Acetone	1:10	3	0	0	0	0
HMX (2.0%)	Acetone	1:100	3	0	0	0	0
HMX (2.5%)	Cyclohexanone	1:10	3	0	0	0	0
HMX (2.5%)	Cyclohexanone	1:100	3	0	0	0	0
HMX (3.3%)	Pure DMSO	1:10	3	0	0	0	0
HMX (3.3%)	Pure DMSO	1:100	3	0	0	0	0
HMX (33.3%)	Pure DMSO	1:10	3	0	0	0	0
HMX (33.3%)	Pure DMSO	1:100	3	0	0	0	0
HMX (3.3%)	Tech grade DMSO	1:10	3	0	0	0	0
HMX (3.3%)	Tech grade DMSO	1:100	3	0	0	0	0
HMX (33.3%)	Tech grade DMSO	1:10	3	0	0	0	0
HMX (33.3%)	Tech grade DMSO	1:100	3	0	0	0	0

a/ 0.5 ml

b/ The Draize Test was used to evaluate skin effects (table VII, p. 27).

3. Discussion.

Sensitization usually involves the entire skin surface. It may occur anywhere on the body but is most easily induced in an area that has been inflamed because of primary irritation, infection, burns, and other causes. It has been postulated that the concentration of lymphocytes in such an area greatly aids or is even necessary for the production of sensitization. It is not the simple chemical haptene itself that acts as the antigen, but its combination with the proteins of the skin form the haptene-protein antigen conjugate. This conjugate then acts as the stimulus that produces the immune response in the host. The antibodies formed are not only directed against the haptene fraction of the conjugate but also against the protein carrier. When a sensitized animal is challenged by the haptene itself (either by topical application or intradermal injection) an inflammatory response is manifested within 24 to 48 hours.

On this basis, it is reasonable to assume that a simple chemical which is a potential allergen will induce sensitization more readily if it is a primary irritant. There are, however, cases where nonirritating substances have produced sensitization, and vice versa, where highly potent primary irritants have failed to produce sensitization. Rothberg[7,2] showed that primary irritants cause sensitization in his studies with guinea pigs which were designed to determine the sensitization potential of α-bromo-α-tolunitrile (CA), 10-chloro-5,10-dihydrophenarsazine (DM), α-chloro-acetophenone (CN) and o-chlorobenzylidinemalononitrile (CS). In these studies both CN and CS were found to be primary irritants, causing extensive erythema, edema, necrosis, and sometimes eschar formation when administered intradermally or percutaneously during the sensitization period. They were also found to be sensitizers.

In our studies, both cyclohexanone and DMSO, with and without RDX and HMX, were found to be irritants when applied topically. Topically applied DMSO was also observed to cause a "smoking" action, an effect obviously due to a chemical reaction with the skin. When administered intradermally, with or without the explosive components, cyclohexanone, DMSO, and acetone caused skin damage at the concentrations used. Yet none of these solvents or solvent RDX-HMX combinations demonstrated a sensitization potential when the animals were later challenged by either route. These findings confirm that a compound can be a skin irritant without being a sensitizer.

Since all solvents invloved in these studies except topically administered acetone caused skin irritation at the concentrations used, it is difficult to determine the contribution of RDX and HMX to irritancy. By the intradermal route any contribution by RDX or HMX was obscured by the solvent effects. The order of solvent irritancy by this route is: (1) cyclohexanone, (2) acetone, and (3) DMSO. This order does not appear to be related to weight of solvent administered per injection because the least effective, DMSO, was administered in the greatest quantity.

The contribution made to skin irritancy by topically administered RDX and HMX in each solvent is also difficult to determine because of the initial erythematous and residual desquamation effects of cyclohexanone and DMSO. Since acetone was not irritating and the residual amounts of RDX and HMX left on the skin after each treatment caused no topical irritation, it would appear that RDX and HMX themselves do not cause irritation. This effect could be due to poor skin absorption of the neat compounds. Patty[1] reports that there is no evidence of skin absorption of RDX. Information on the skin absorption of HMX is not available.

In the topical sensitization exposures reported here, RDX and HMX were used with the solvents at the highest concentrations obtainable. RDX and HMX were also tested by intradermal injection which is even more direct and reliable than topical testing.

Our data show that neither RDX nor HMX cause sensitization by either exposure route when tested by the method of Landsteiner and Chase.[69,70,71] Also, none of the solvents used in these studies produce sensitization.

In a report of primary irritancy and sensitization dermatitis, particularly of the face and eyelids during the nitration process of RDX, it was shown that an unidentified component in the fumes from the reaction mixture was responsible.[7] McConnell[8] attributed some dermatitis to the manufacture of RDX, but this was probably related to intermediates because significant dermatitis was not observed in individuals handling the purified material. This observation was corroborated by Von Oettingen's[3] findings that patch testing with the moistened solid did not produce irritation.

Sunderman[66] showed that powders of both RDX and HMX caused primary irritancy in humans when patch tested as described by Swartz and Tulipans.[73] With this method he also demonstrated a negative human sensitization potential for RDX but a positive sensitization potential for HMX. RDX is manufactured by the nitration of hexamethylenetetramine which is obtained by the reaction of formaldehyde and ammonia.[74] Formaldehyde is liberated from this reaction and is oxidized by the nitric acid if the mixture is allowed to stand. If the formaldehyde remains in the spent acid after drowning, there is difficulty in recovering the nitric acid from the spent solution. As a result of the rupture and degradation of the hexamethylenetetramine molecule, numerous aliphatic and cyclic nitro compounds are present in crude RDX. The most important is cyclotetramethylenetetranitramine, or HMX. Since HMX is a byproduct of RDX and has the same basic molecule and group types, it is difficult to understand why RDX would not, and HMX would, cause sensitization. As a general rule, if a parent compound is a true sensitizing agent, most of the intermediates involved in its synthesis are also sensitizers. A good example of this is the intermediates involved in dye production.[75]

It was not within the scope of this study to determine the intradermal or cutaneous toxicity of RDX or HMX in guinea pigs. However, since several animals (5/12) died from the topical application of one or two 0.5-ml applications of 33% HMX in pure or technical grade DMSO, a comment is in order. Even though five animals died from HMX-DMSO exposures, seven others were able to withstand, without symptoms, nine such treatments administered at 48 to 72-hr intervals over 19 days. Also, in the studies shown in table XIII a single dose of 2 gm/kg and three 1 gm/kg doses did not kill any guinea pigs.

The lowest single dose to cause death was 0.465 gm/kg. If the human were comparable to the guinea pig in toxicologic response to HMX-DMSO administered topically, the lethal dose would be approximately 33 grams HMX (70 kg man) in 100 ml of DMSO. This dose is based on the assumption that HMX-DMSO was uniformly spread over a large surface area of skin and that no effort to remove it was made. This is a considerable amount of HMX and when compared to other percutaneously potent compounds, it must be considered relatively nontoxic.

Thirty-three percent RDX-DMSO showed no toxic effects when applied in the same amounts and over the same period of time.

4. Summary of Topical, Intradermal and Sensitization Studies of Solutions of RDX and HMX, and of Solvents.*

Under the experimental conditions described in this study, the following can be said.

1. No deaths occured in guinea pigs given repeated topical doses of 5.4% RDX-acetone, 7.5% RDX-cyclohexanone, or 33% RDX-DMSO.

2. No deaths occurred in guinea pigs given repeated topical doses of 2.0% HMX-acetone, 2.5% HMX-cyclohexanone, or 3.3% HMX-DMSO. Several deaths occurred after one or two exposures to 33% HMX in DMSO. The deaths were attributed to HMX because no deaths were produced by neat DMSO.

3. Topically applied DMSO caused greater skin damage than cyclohexanone. Solutions of RDX and HMX in these solvents did not produce noticeably greater skin damage than the solvents themselves. No visible damage was caused by topical applications of solutions of RDX and HMX in acetone.

4. Cyclohexanone, acetone, and DMSO, administered intradermally, with or without RDX and HMX, caused severe skin damage. This prevented a reliable assessment of the effects of RDX and HMX by this route.

5. Acetone, cyclohexanone, and DMSO did not produce skin sensitization in guinea pigs when administered intradermally or topically.

6. Solutions of RDX or HMX did not produce skin sensitization in guinea pigs when administered intradermally or topically.

E. Cataracts Found in Guinea Pigs Following Cutaneous and Intradermal Applications of Solvents and Solutions of RDX and HMX.*

1. Procedures.

The eyes of 210 guinea pigs were examined after they had received cutaneous or intradermal applications of DMSO (pure and technical grade), acetone, or cyclohexanone. In some of the applications, the solvents contained RDX or HMX. For intradermal injections, the solvent or a solution of explosive in solvent was mixed 1:1 (v/v) with saline (table XIX).

The materials were administered three times a week for 3 weeks to 116 male and 94 female 9- to 18-week old animals. Examinations were made 26 to 110 days after application.

2. Results.

Microscopic examination, using an ophthalmoscope, revealed cataracts in 20% of the animals. The appearance of one typical cataract as revealed by a slit-lamp photograph and a photomicrograph is shown in figures 1 and 2.

* This investigation was conducted by MAJ Roy H. Rengstorff, Medical Research Division, John F. Callahan, Toxicology Division, and PFC William Webb, Medical Research Division

Table XIX. Cataracts Found in Guinea Pigs

Route	Solutions used	Cataracts found in guinea pigs			
		DMSO (pure)	DMSO (tech)	Acetone	Cyclohexanone
Cutaneous	0.5 ml solvent mixed with:	1/4	2/4		
	HMX (%) 33.0	0/6	1/6		
	3.3				
	2.5				0/6
	2.0			0/6	
	RDX (%) 33.0	3/6	1/6		
	7.5				1/6
	5.4			1/6	
	Solvent only (0.5 ml)	3/12	2/12	1/12	1/12
	TOTAL	7/28	6/28	2/24	2/24
	PERCENT	25%	21%	8%	4%
Intradermal	0.5 ml, 1:1 solvent/saline mixed with:				
	0.25% HMX	0/6	2/6	2/6	1/6
	0.25% RDX		0/6	1/6	1/6
	0.05 ml, 0.5% solvent in saline	2/4	0/4	2/4	0/4
	0.05 ml, 1:1 solvent/saline	3/12	4/12	5/12	1/12
	TOTAL	5/22	6/28	10/28	3/28
	PERCENT	23%	21%	36%	11%
Both groups	TOTAL	12/50	12/56	12/52	4/52
	PERCENT	24%	21%	23%	8%

NOTE: Cataracts were bilateral in all animals and resembled those in figure 1 in 15% of the animals. Another 5% had cataracts which were less obvious, appearing as isolated opacities.

Figure 1. Slitlamp Photograph of Guinea Pig Eye 40 Days after Receiving Intradermal Application of 0.05 Ml Pure DMSO in Saline Three Times a Week for Three Weeks. Vacuoles (V) can be seen in the crystalline lens periphery, partly covered by the iris (I). Magnified 12X.

Of the 98 animals that received HMX and RDX in solvents, 16% developed cataracts. The remaining 112 animals received only solvent, and 23% had cataracts. The common factor for both groups was the solvents, and the explosives did not appear to increase the probability of cataracts.

3. Discussion.

A variety of substances, fed or injected, can cause cataracts in experimental animals. These include naphthalene, iodoacetate, Myleran (1-4-butanediol dimethanesulfonate), Mimosine (3-hydroxy-4-oxo-1(4H)-pyridinealanine), dinitrophenol, certain quinoid substances, ergot, thallium, adrenaline, morphine-like drugs, and DMSO. In a cursory search of the literature, no reports of cataracts caused by acetone and cyclohexanone have been found.

In a separate study in this laboratory, small multiple doses of acetone, cyclohexanone, and dimethyl sulfoxide administered either topically or subcutaneously on the backs of guinea pigs over a period of 3 to 8 weeks caused cataracts in 29 out of 120 animals. Lens changes began as early as 8 weeks and as late as 6 months. They consisted of subcapsular focal or extensive vacuolated areas extending from the periphery towards the center of the lens. The histological appearance of the lenses was similar to that of senile cataracts and some forms of diabetic cataracts.[65]

ANTERIOR SURFACE

POSTERIOR SURFACE

Figure 2. Photomicrograph of the Crystalline Lens Shown in figure 1. The abnormality appears anteriorly and posteriorly as exaggerated tissue vacuoles (V) involving the entire subcapsular region. CA – capsule. Magnified 80X.

IV. RESUME

A. Intravenous Effects of RDX, HMX, and Three Solvents.

1. Intravenous Toxicity of RDX and HMX in Mice and Guinea Pigs.

The intravenous LD50 for mice of RDX in DMSO is 18.7 (15.7-22.3) mg/kg and of HMX in DMSO is 28.9 (25.1-33.3) mg/kg. Deaths occurred in 5 to 10 minutes and were preceded by mild convulsions and labored breathing. The survivors were lethargic for several hours thereafter, but were normal within 24 hours.

The intravenous LD50's in guinea pigs for RDX and HMX in DMSO are 25.1 (20.0-31.6) mg/kg and 28.2 (20.2-39.8) mg/kg, respectively. Convulsions, prostration, and deaths occurred within 5 minutes.

2. Intravenous Toxicity in Dogs.

The intravenous administration of about 0.1 ml/kg of acetone produced significant but transient decreases in blood pressure. The same dose of cyclohexanone caused a transient cardiac arrest. Cyclohexanone also produced high-voltage, low-frequency patterns in the EEG. This is typical of central nervous depression, or of sleep. During this phase, the animals were semicomatose and pain responses were depressed. These solvent effects obscured the actions of dissolved RDX or HMX. The only effect of 0.1 ml/kg DMSO was an appreciable but transient decrease in blood pressure.

The intravenous administration of 40 mg/kg of RDX in DMSO was followed in less than 15 seconds by an epileptic-type, spike-and-dome EEG pattern and concomitant convulsive movements. Changes in blood pressure and heart rate were negligible at this time. Two dogs died, apparently in cardiovascular collapse, at 45 and 90 minutes after injection of the material. Twenty mg/kg of RDX in DMSO caused the CNS effects but not death. (The convulsive-type EEG pattern and muscle activity could be precipitated by photic or tactile stimuli.)

The intravenous administration of 40 mg/kg of HMX in DMSO caused death in cardiovascular collapse in 1 and 3 minutes in two dogs; two others died in about 14 hr. When two 20 mg/kg doses were given 30 minutes apart to two additional dogs, there was an immediate fall in blood pressure and one dog died in 1 min. Epileptic-type EEG and muscle movements developed in the other dog after several hours. It died 14 hours after an apparent recovery from the central and cardiovascular effects.

A prominent difference between RDX and HMX in the dog was the time of appearance of the central nervous system effects. The epileptiform activity occurred almost immediately with RDX but after a delay with HMX.

B. Local Effects of Topical Applications.

The three solvents and solutions of RDX and HMX in the solvents occasionally cause erythema and signs of irritation or pain when applied to the skin of rabbits, guinea pigs, or dogs.

DMSO is the least volatile and possibly the most active on the skin. Acetone, which is the most volatile, was the least active. DMSO sometimes caused fine wisps of smoke to rise from the skin surface. Also, the animals vocalized, squirmed, licked the site of application, and exhibited reactions indicative of pain. These skin reactions are in accord with those previously described for DMSO in animals and man.

Following repeated application, DMSO and cyclohexanone caused the skin of rabbits, dogs, and guinea pigs to become dry and hard. A slight flaking or desquamation occurred in the outer layers. There were no fissures, eschars, or scab formations.

C. Systemic Effects Following Topical Skin Application.

1. Blood Values in Rabbits.

Single or repeated topical skin application to rabbits of 1.0-ml doses of DMSO, cyclohexanone, or acetone, or solutions of RDX and HMX in the three solvents did not significantly alter the red blood cell count, the white blood cell count, hematocrit, hemoglobin, blood urea nitrogen, creatinine, alkaline phosphatase, serum glutamic oxalacetic transaminase nor electrolytes.

2. Organ and Tissue Examination in Rabbits.

No gross or microspic abnormalities were found in the organs or tissues of rabbits that died or were sacrificed following repeated topical applications of the solvents or solutions of RDX or HMX in the three solvents.

3. Physiological Parameters in Dogs.

Single and repeated topical applications of the three solvents or of saturated solutions of RDX or HMX in the solvents to unanesthetized dogs produced no significant changes in blood pressure, heart rate, EKG or EEG patterns, or respiration. There were no abnormal gross or EEG responses to visual, auditory, tactile, irritant, or painful stimuli. Doses of RDX and HMX were as great as 480 mg/kg repeated on 3 consecutive days.

Since intravenous doses of 20 to 40 mg/kg of RDX or HMX produced marked toxicological responses in dogs, it is indicated that the canine skin is poorly penetrated by these explosives.

4. Lenticular Opacities.

As mentioned in our review of the literature, lenticular opacities have been produced by DMSO administered by various routes of administration, including percutaneous, in various animal species,[14,18,47] but not in man.[35,38,40,42,43,49] This ocular effect was noted following topical and intradermal application of DMSO, acetone, and cyclohexanone to guinea pigs in the present study. To the knowledge of the present investigators, lenticular effects have not been attributed previously to these solvents. The number of animals was too small to rank the solvents for this effect; however, it was indicated that DMSO was the most damaging and cyclohexanone was the least injurious. Also, it was indicated that the presence of RDX or HMX was no more damaging than the solvents alone. Subsequent studies in these laboratories to induce lenticular opacities in rabbits with acetone have not been successful. *

* Rengstorff, R. H., Petrali, J. P., and Sim, V. M. Unpublished Data.

Although lenticular actions due to these solvents have not been seen in man, it would be injudicious to minimize human exposure.

5. Toxic Signs and Death in Rabbits, Guinea Pigs, and Dogs.

No toxic signs or deaths resulted in rabbits, guinea pigs, or dogs from the single or repeated topical skin application of:

DMSO, acetone, or cyclohexanone

RDX in DMSO

HMX in acetone or cyclohexanone

Paralysis in rabbits, convulsions in guinea pigs, and some deaths in both species occurred following topical application. One rabbit died after the fifth 0.1-ml dose and one after the tenth application of the 1.0-ml dose of 5.4% RDX in acetone. Another rabbit died after the eighth 1.0-ml application of 7.5% RDX in cyclohexanone. Six rabbits died after repeated 1.0-ml doses of 33% HMX in DMSO: three after the second dose, one after the fifth dose, one after the sixth dose, and one after the twentieth dose. Five of 12 guinea pigs died 24 to 48 hr after topical exposure to 33% HMX in DMSO, four after single doses of 465, 477, 507, and 546 mg/kg, and one after two doses of 1126 mg/kg.

In another abbreviated test to find the percutaneous LD50, no deaths in guinea pigs occurred with RDX or HMX in DMSO at doses from 316 to 2,000 mg/kg. Thus, although some deaths occurred in guinea pigs at single doses of 465 to 546 mg/kg, the LD50 may be above 2000 mg/kg. Following three 1000 mg/kg doses of 33% HMX in DMSO, guinea pigs became apprehensive and lost weight; their skin became spongy and absorbent after each application and lost its normal color.

Convulsions preceded death in guinea pigs. Lethality could possibly be attributed to excitation of the central nervous system. It is not known whether or not deaths in rabbits were preceded by signs of excitation of the central nervous system. Paralysis, attributed to broken backs, preceded death in rabbits. It is possible that the backs were broken during convulsive episodes which occured during the night or during other periods when the animals were not being observed. It is questionable that the deaths were attributable to the agents.

Central excitation has been noted in the electroencephalographic pattern in dogs. This information indicates that HMX in DMSO can penetrate the skin of some mammals in sufficient dosage to produce toxic signs and death. Very high doses are needed.

D. Sensitization

Skin application of the three solvents or of RDX or HMX in the solvents, followed by topical skin or intradermal challenge with the same material, yielded no evidence of sensitization.

V. CONCLUSIONS.

1. Acetone and cyclohexanone in sufficient dosages caused some drying and hardening of the skin (locally), and central and cardiovascular depression systemically. DMSO

caused the above mentioned cutaneous effects. All three solvents produced some lenticular changes. None of the solvents influenced blood values or histological appearance of various tissues. None of the solvents caused sensitization.

2. DMSO was the strongest and acetone the weakest in producing local skin effects when applied topically.

3. DMSO was the strongest and cyclohexanone the weakest in causing lenticular opacities.

4. HMX in DMSO penetrated the skin of rabbits in sufficient quantities to produce convulsions, paralysis, and death only when applied in very large doses.

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APPENDIX A

FIGURES

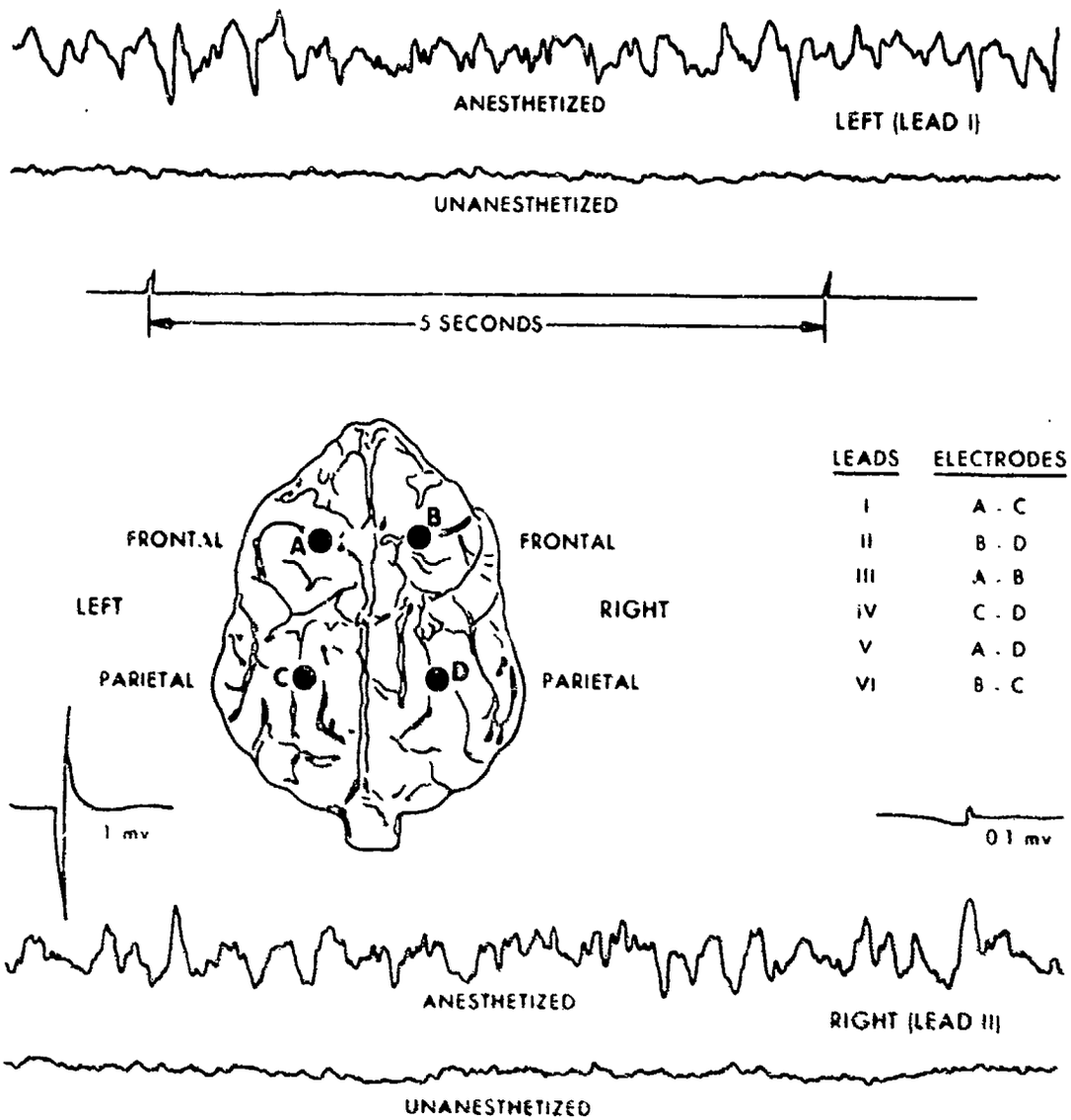

Figure A-1. Diagrammatic Sketch of Brain Showing Approximate Placement of Electrodes. Sample Tracings of EEG in Anesthetized and Unanesthetized Dog.

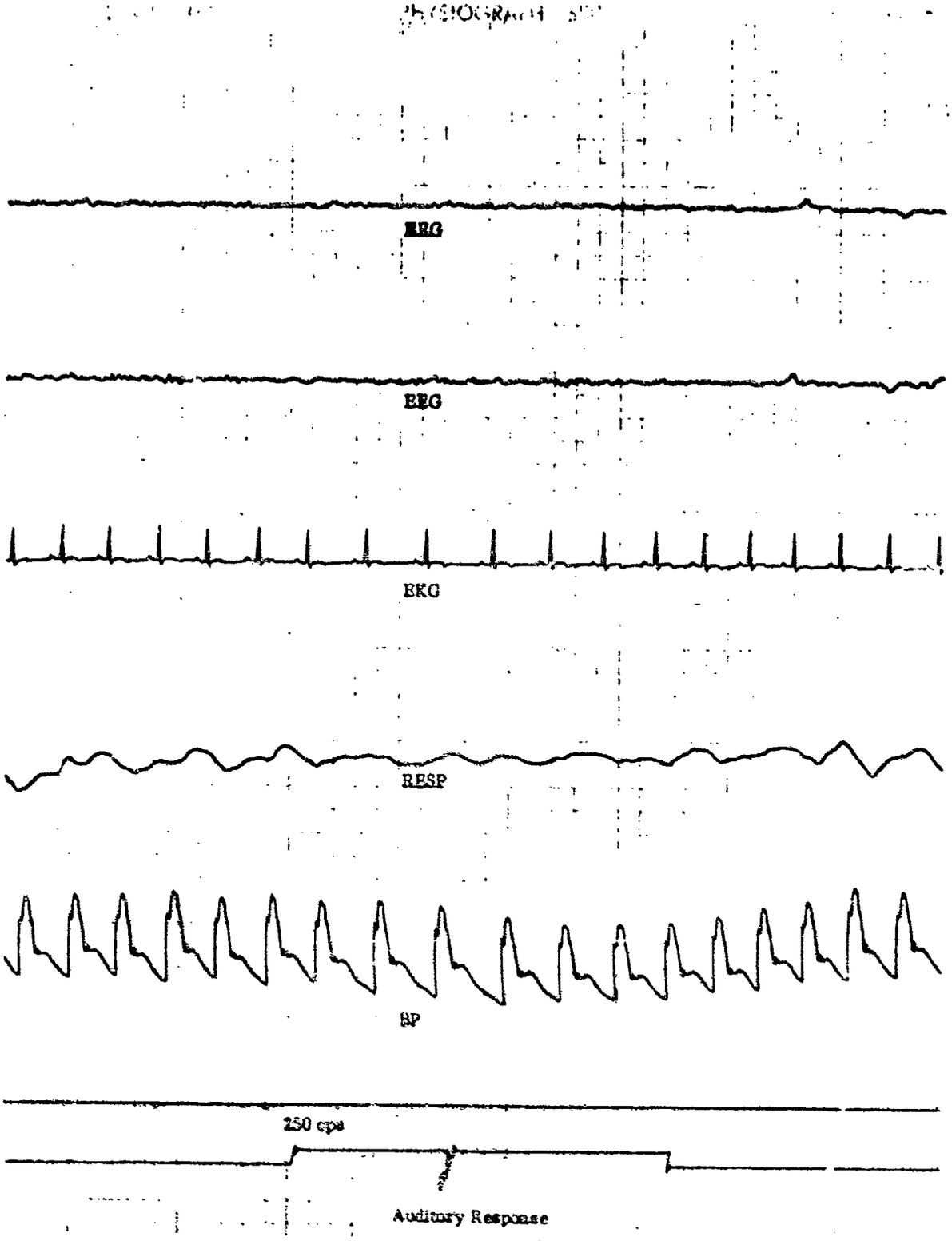

Figure A-2. Typical Responses Obtained from Various Physiologic Parameters of Dog after Noise (250 cps)

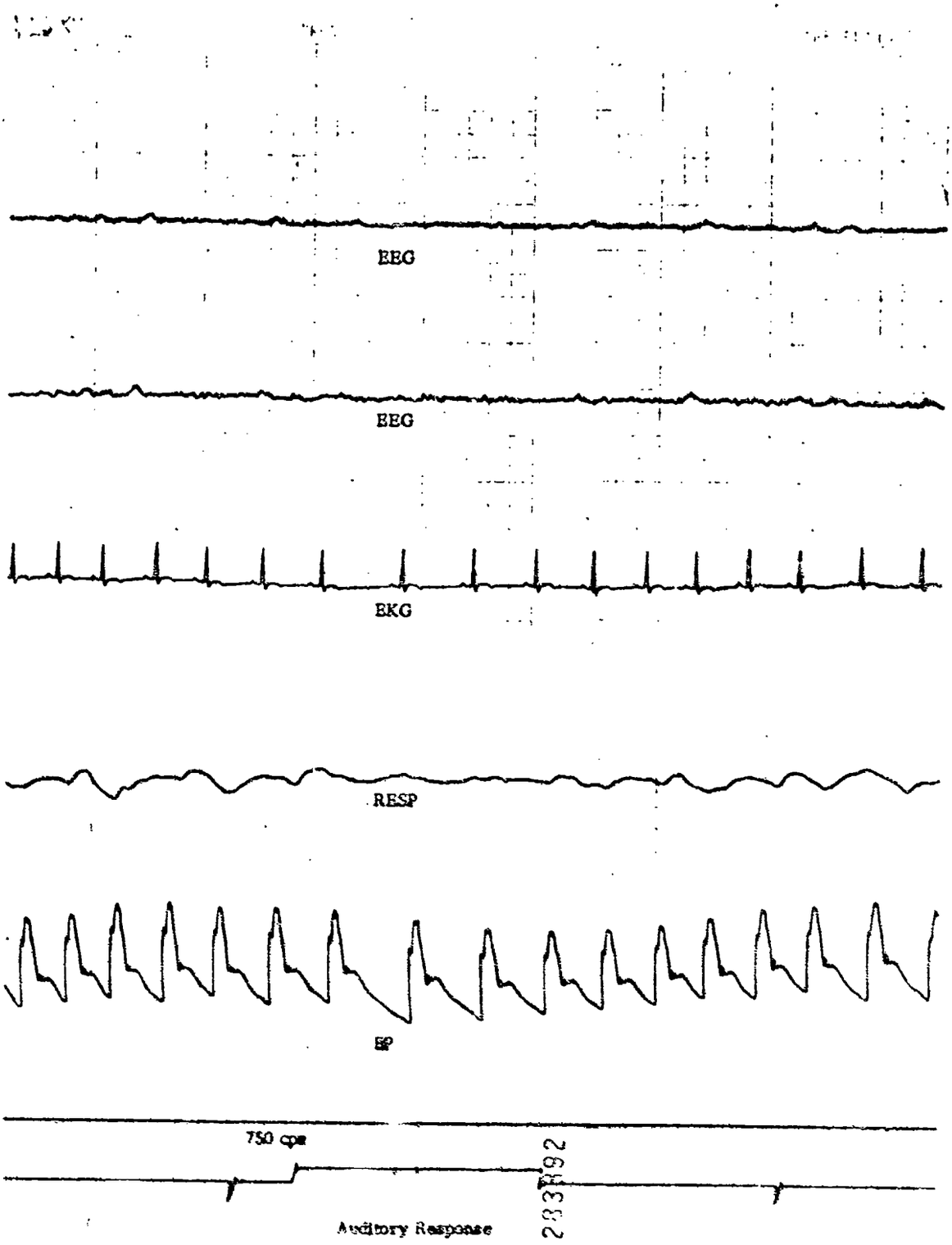

Figure A-3. Typical Responses Obtained from Various Physiologic Parameters of Dog after Noise (750 cps)

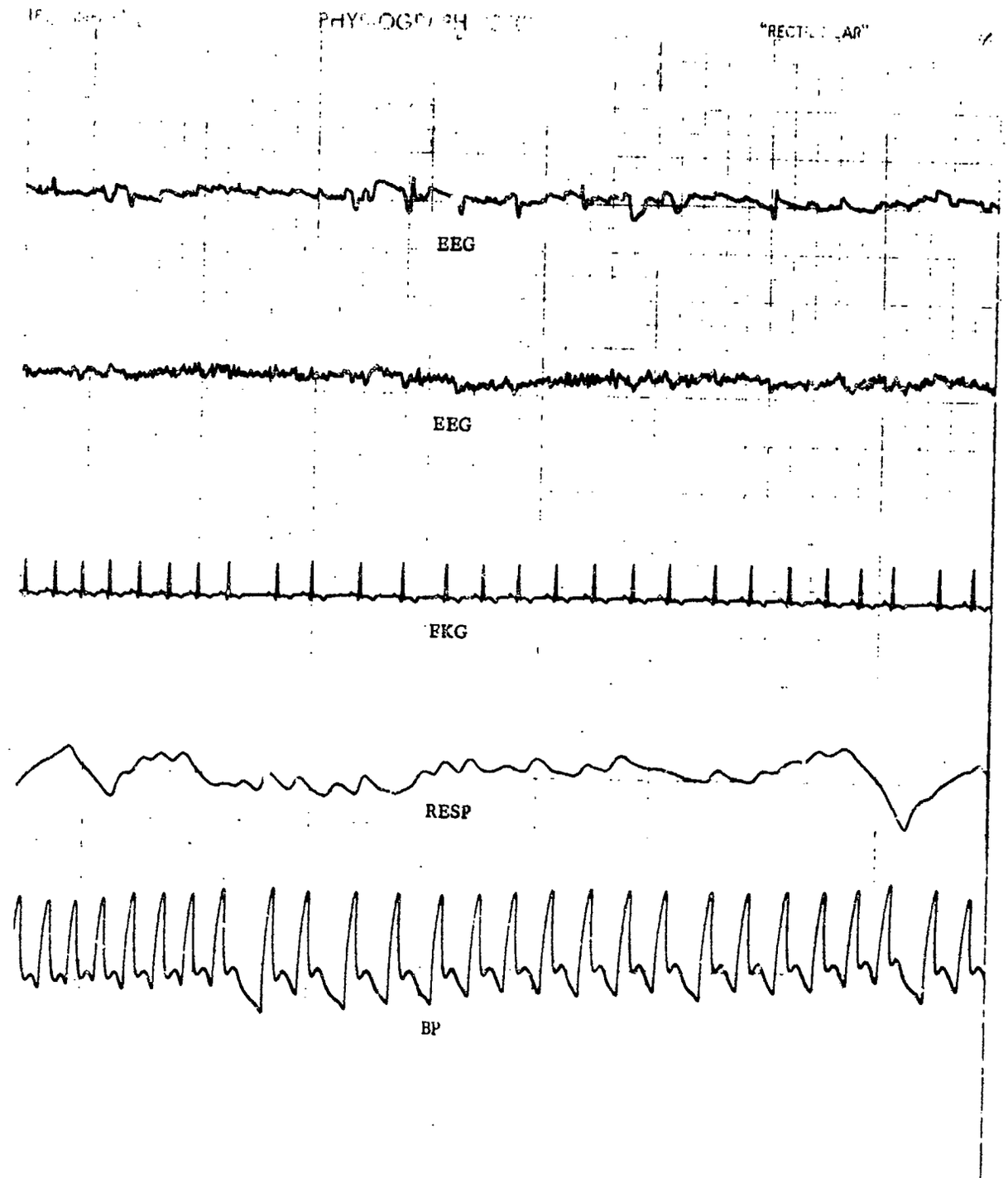

Figure A-4. Typical Responses Obtained from Various Physiologic Parameters of Dog after Nasal Stimulation

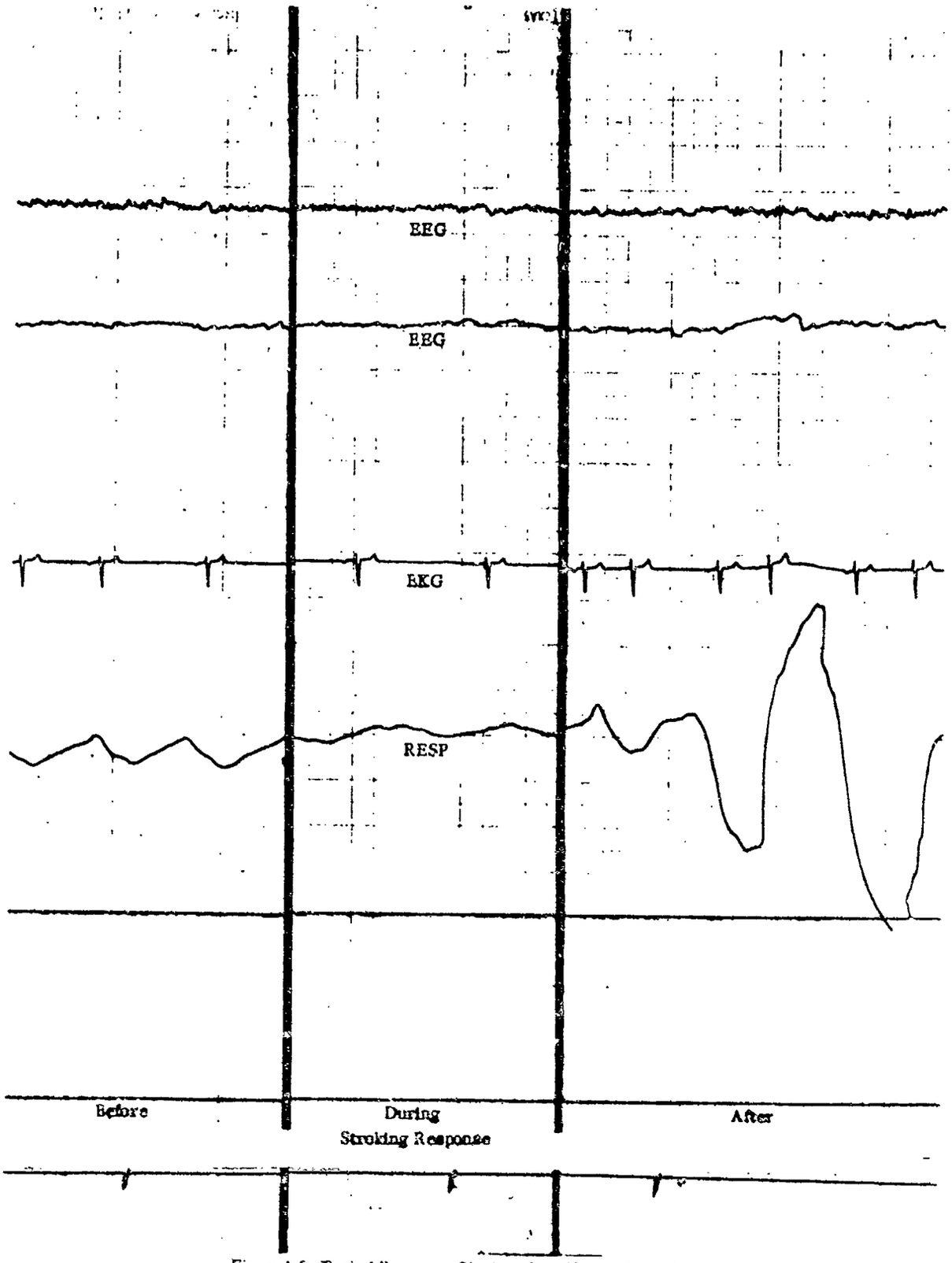

Figure A-5. Typical Responses Obtained from Various Physiologic Parameters of Dog after Stroking

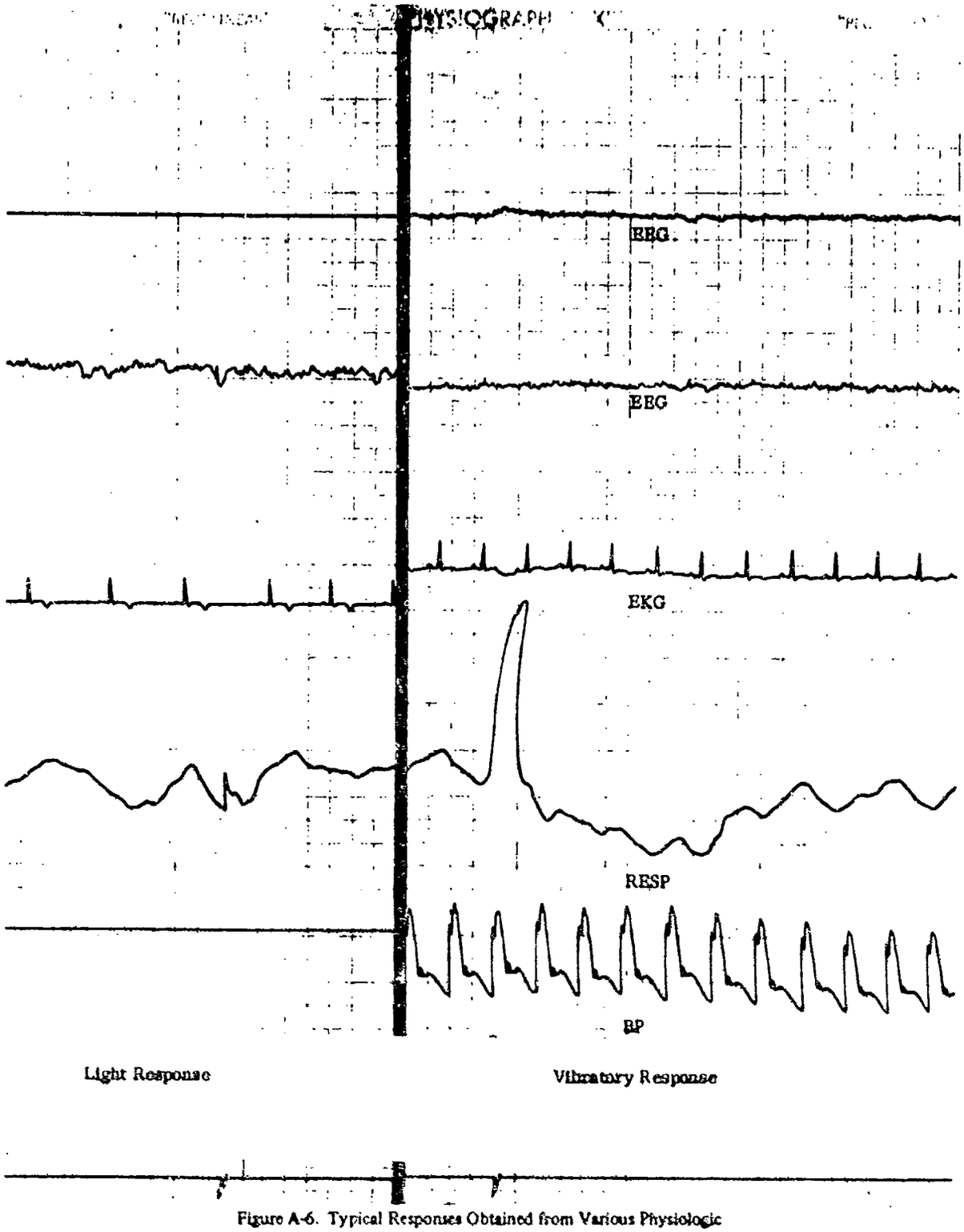

Figure A-6. Typical Responses Obtained from Various Physiologic Parameters of Dog after Exposures to Light and Rapping

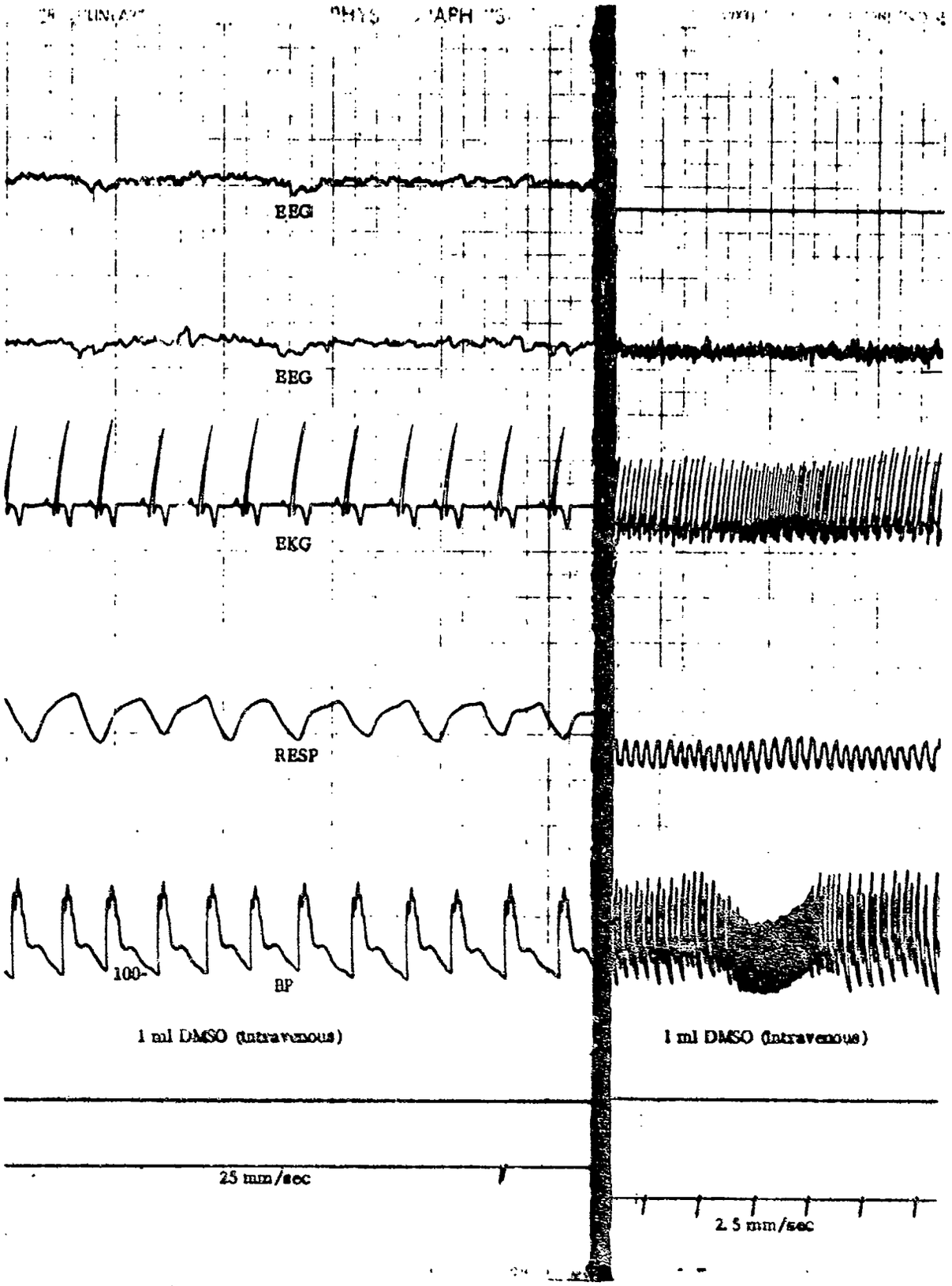

Figure A-7. Effects of DMSO Upon Physiologic Parameters of Unanesthetized Dogs

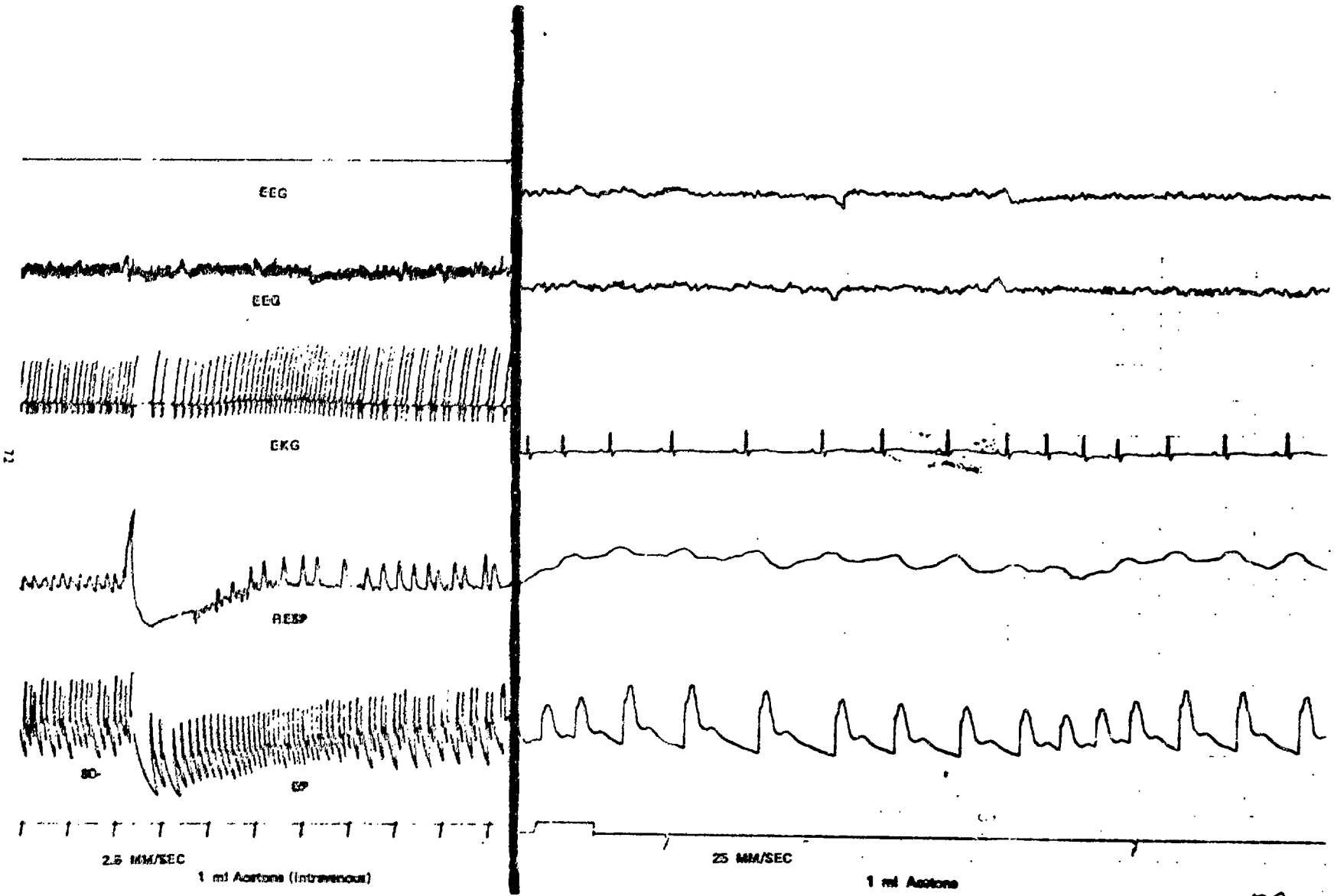

Figure A-6. Effects of Acetone Upon Physiologic Parameters of Unanesthetized Dogs

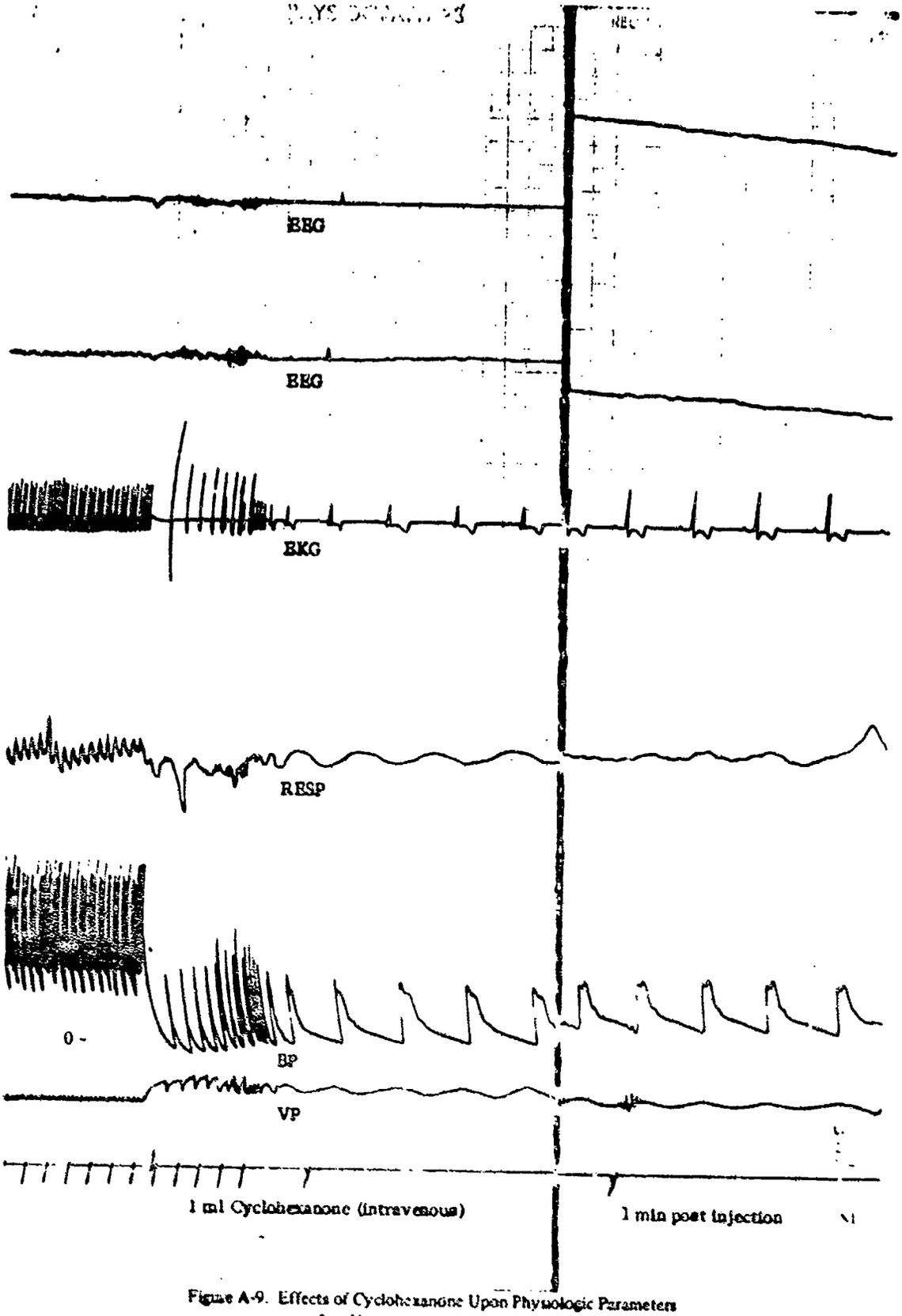

Figure A-9. Effects of Cyclohexanone Upon Physiologic Parameters of an Unanesthetized Dog (1 min)

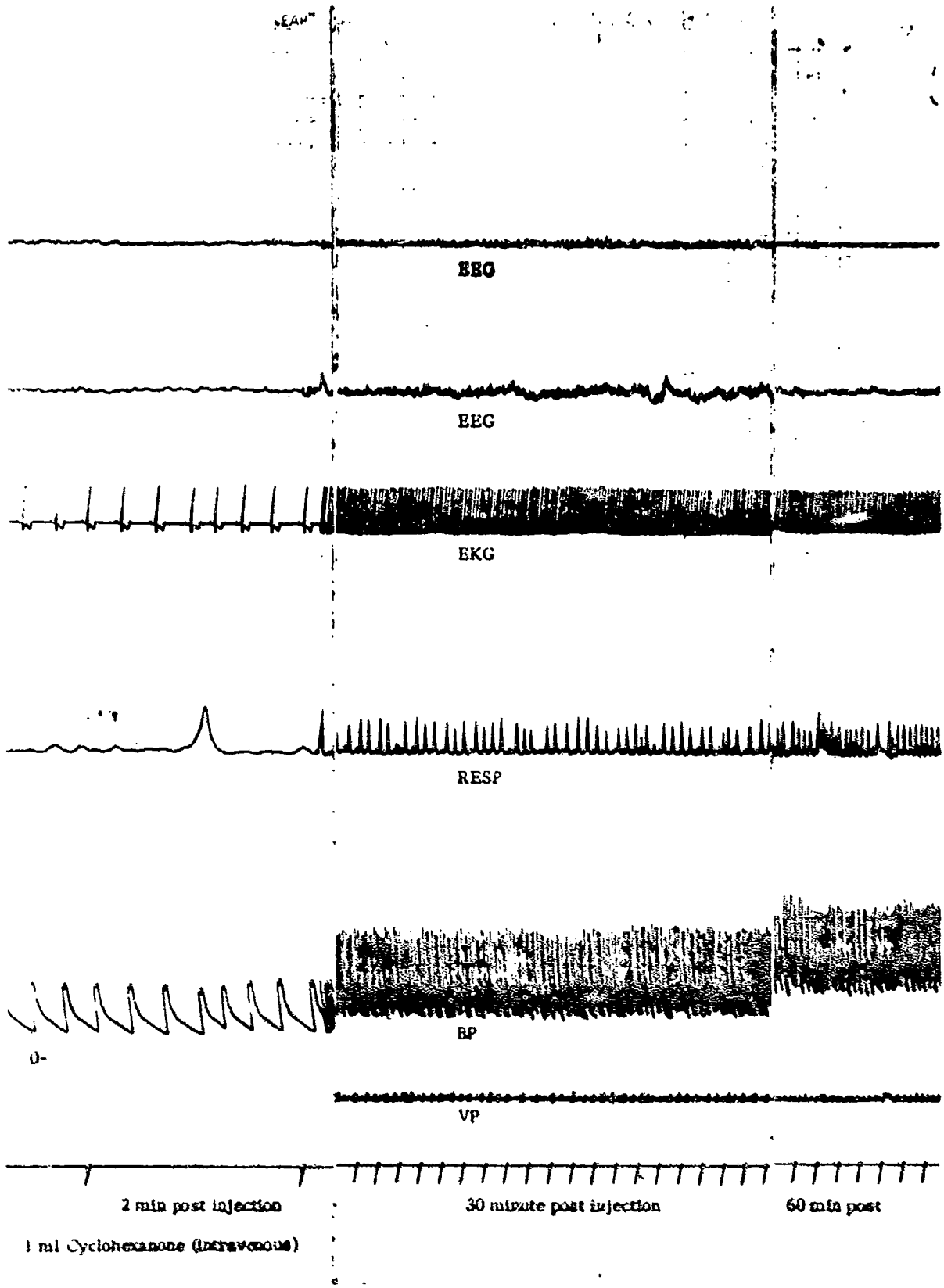

Figure A-10. Effects of Cyclohexanone Upon Physiologic Parameters of an Unanesthetized Dog (2, 30, and 60 min)

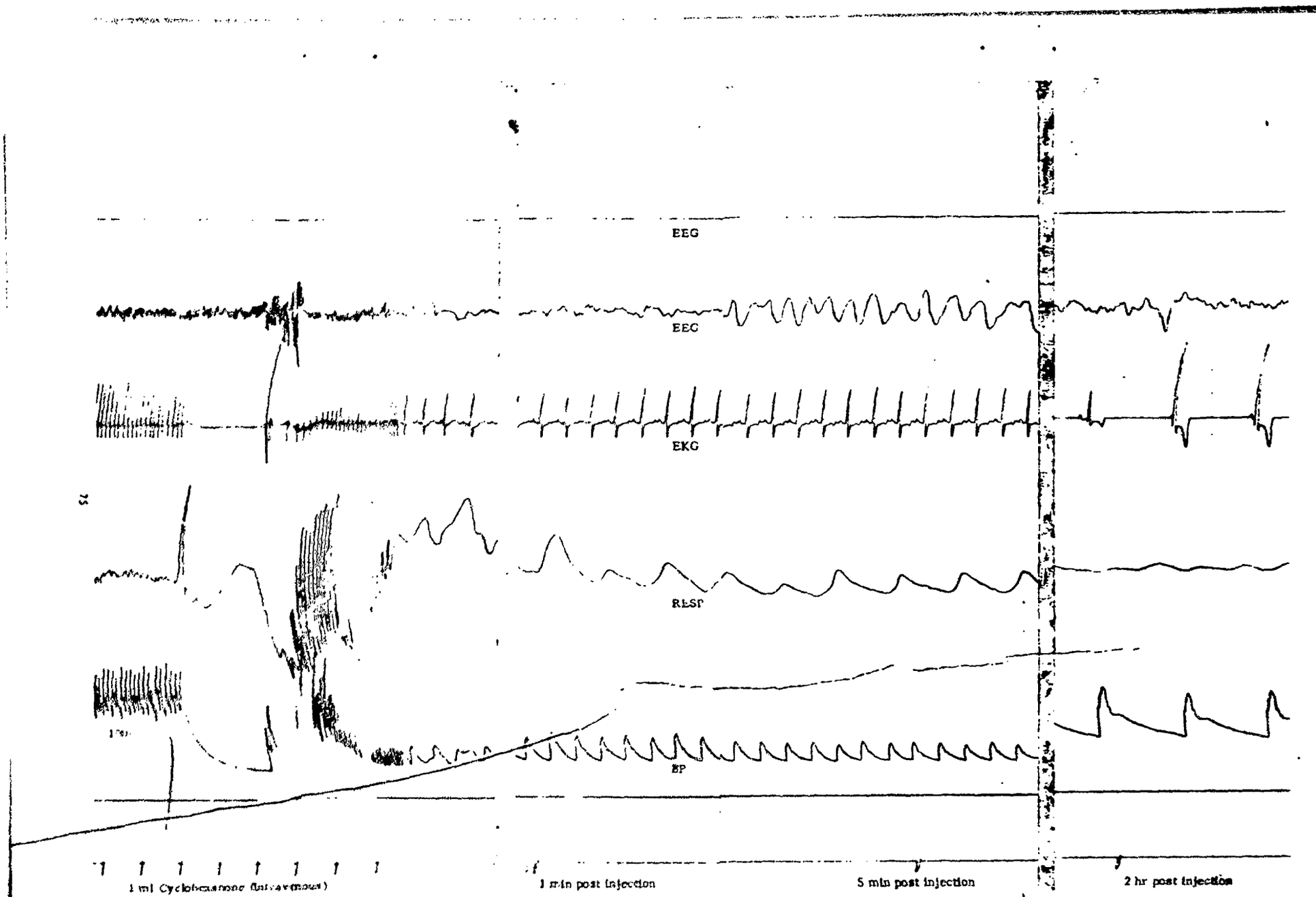

Figure A-11 Effects of Cyclohexanone Upon Physiologic Parameters of an Unanesthetized Dog (1 and 5 min. and 2 hr)

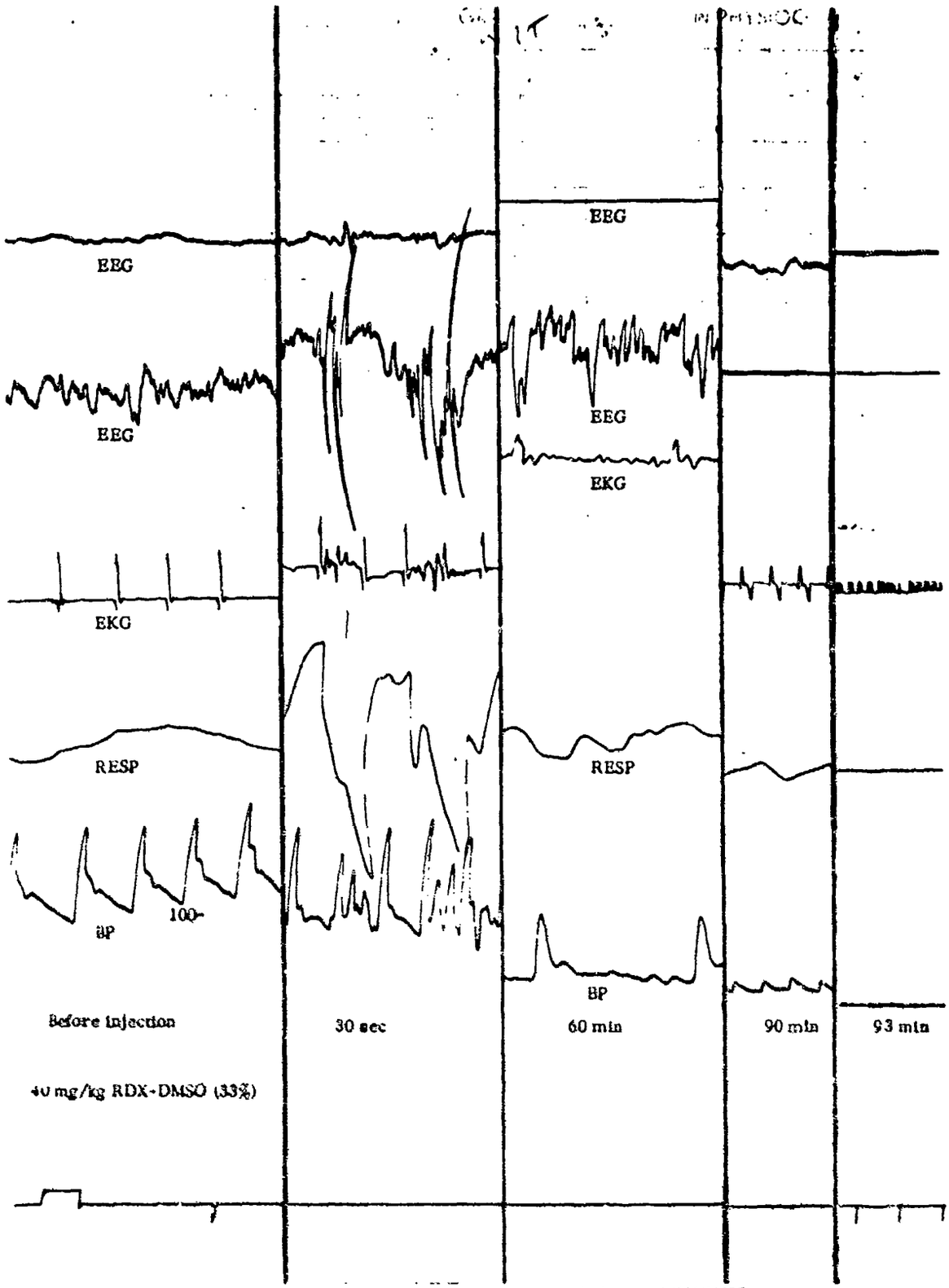

Figure A-12. Effects of Intravenous Administration of 40 mg/kg RDX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog

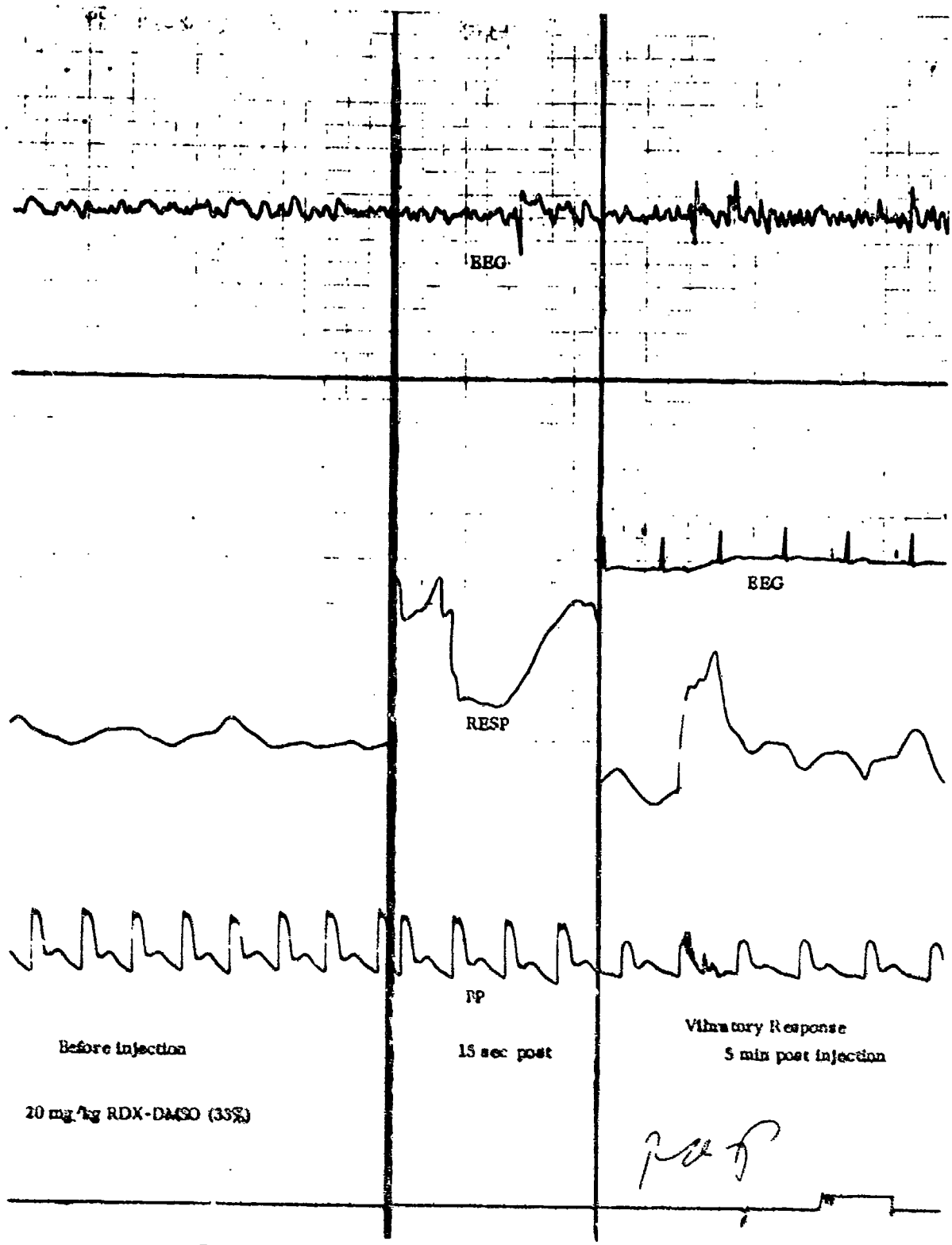

Figure A-13. Effects of Intravenous Administration of 20 mg/kg RDX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog

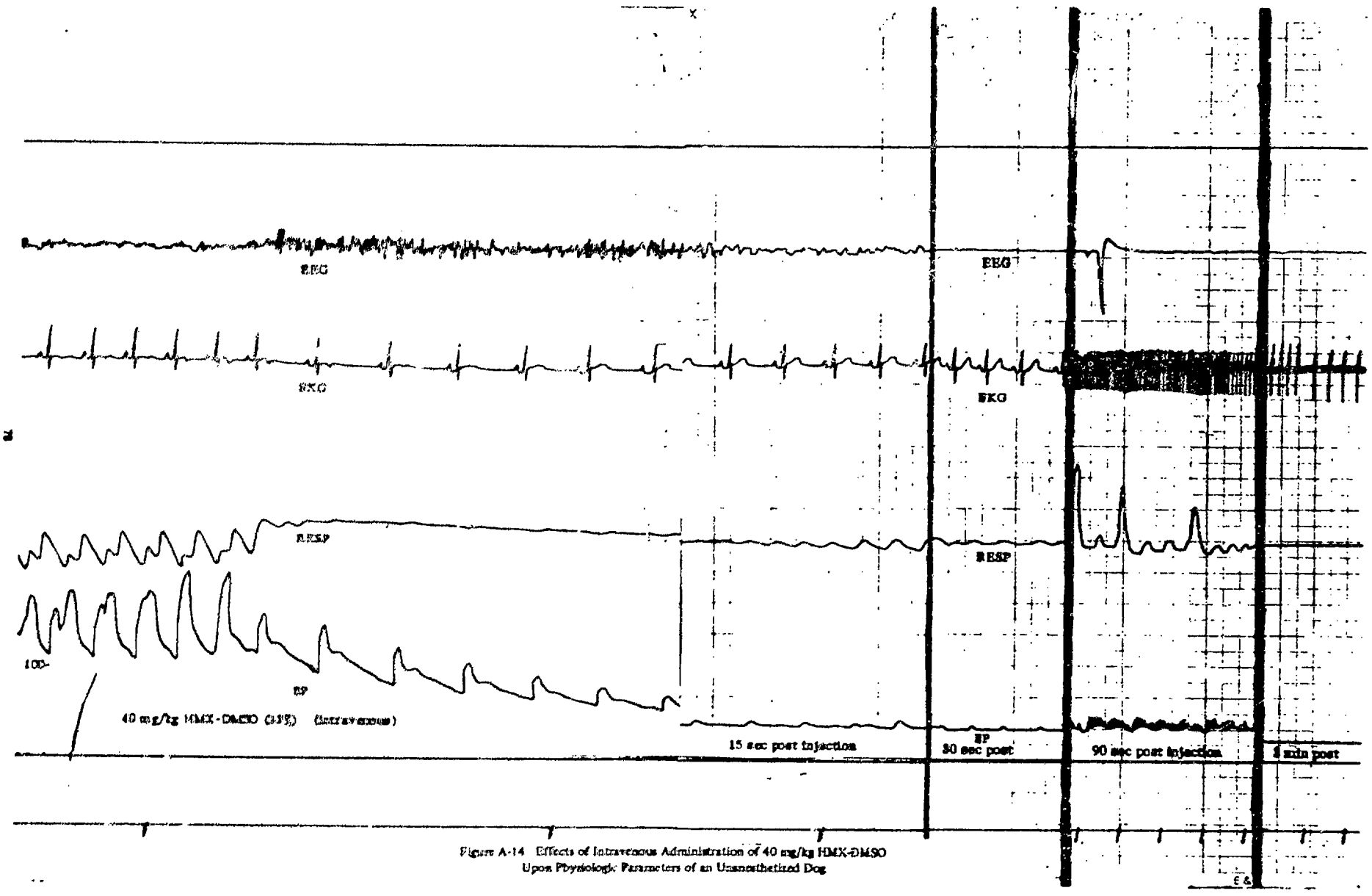

Figure A-14 Effects of Intravenous Administration of 40 mg/kg HMX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog

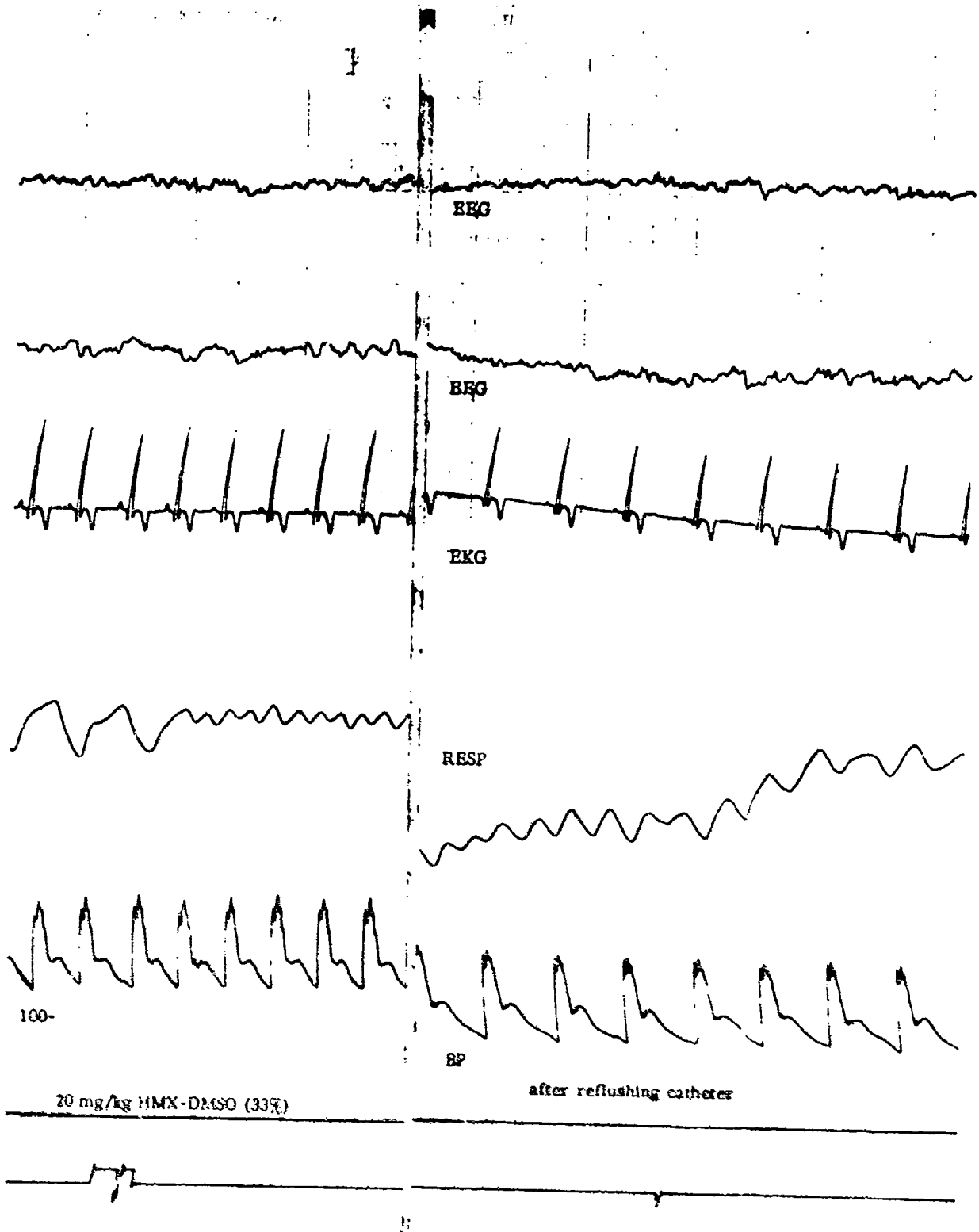

Figure A-15 Effects of Intravenous Administration of 20 mg/kg HMX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog (after reflushing catheter)

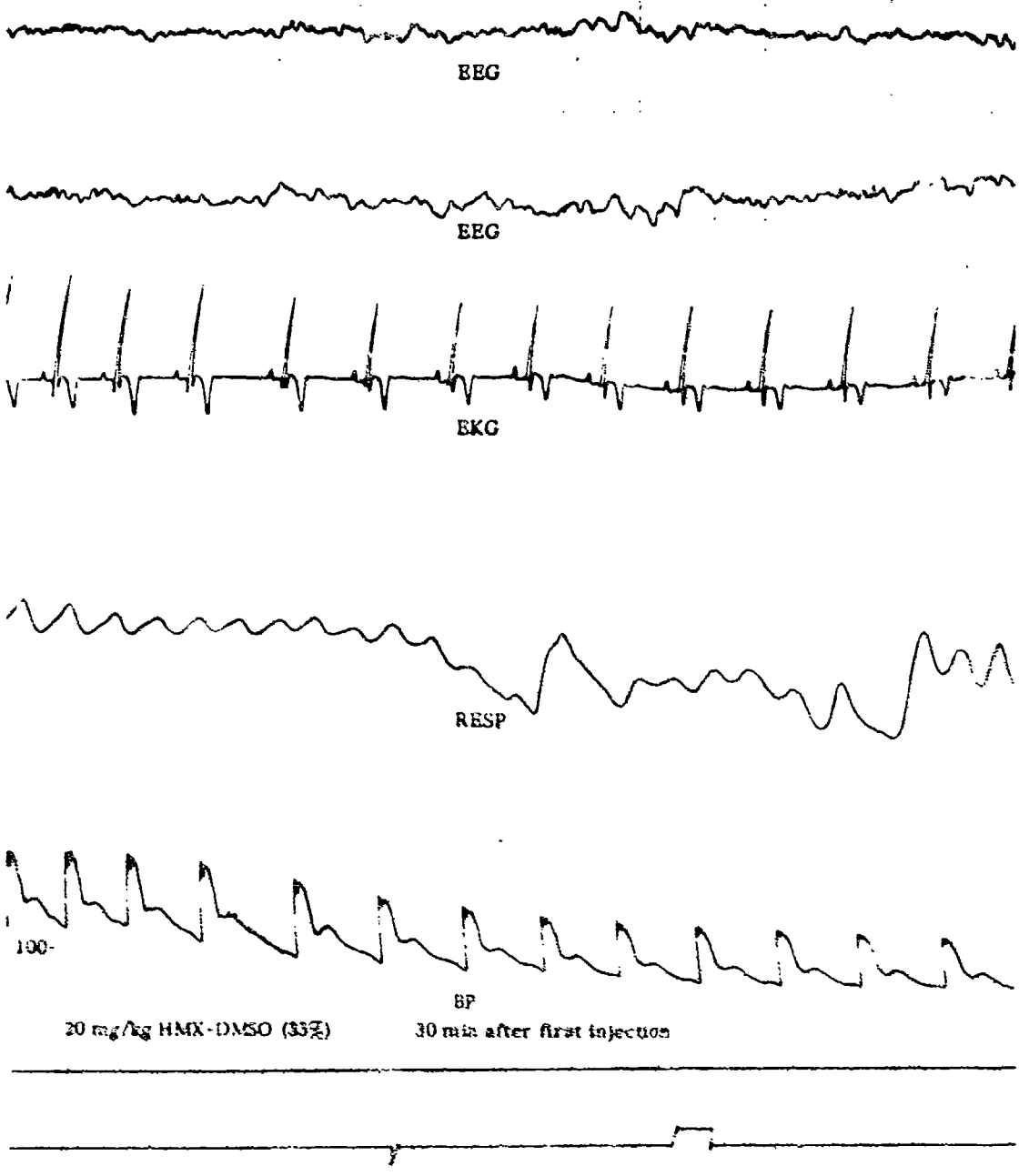

Figure A-16. Effects of Intravenous Administration of 20 mg/kg HMX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog (30 min)

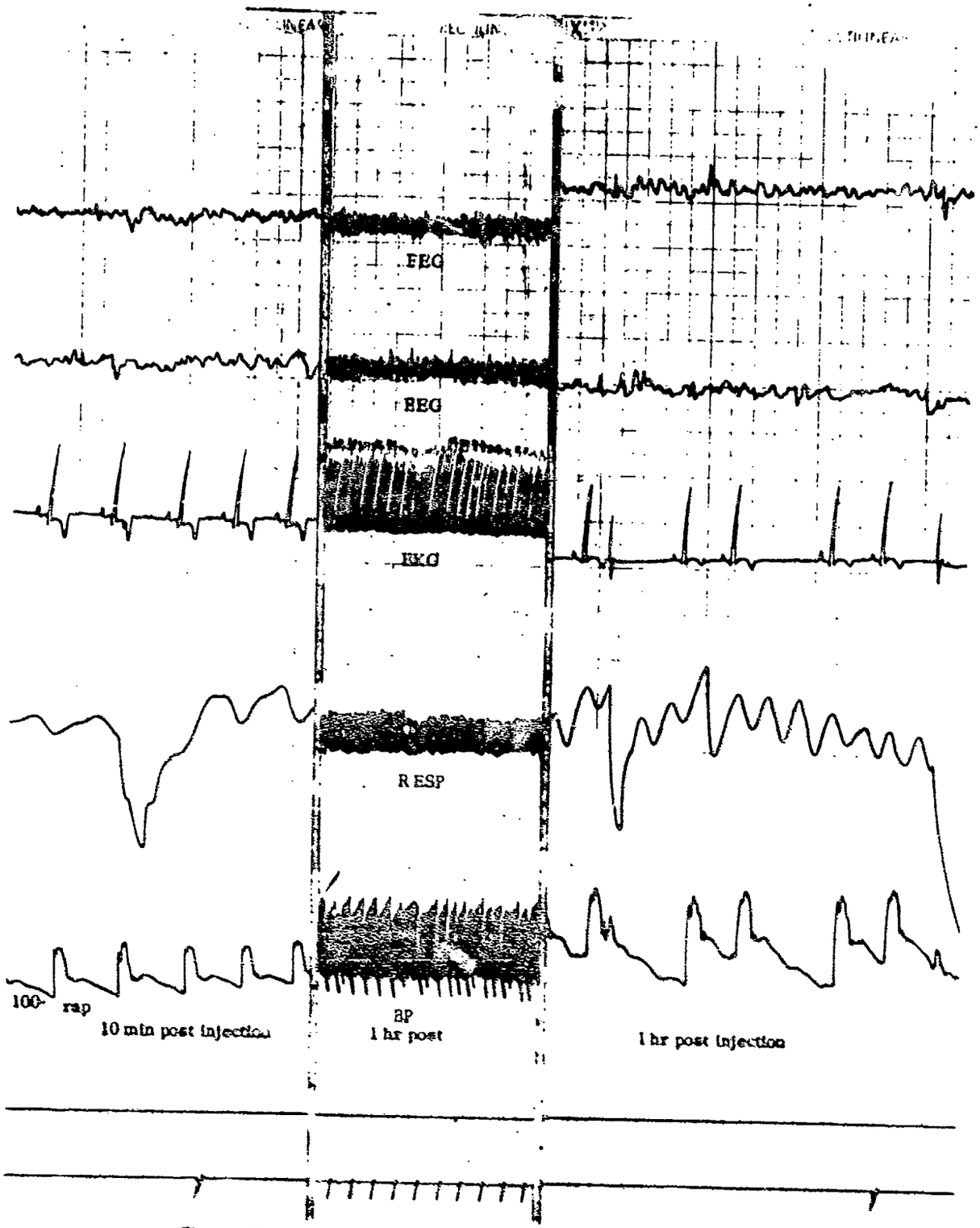

Figure A-17. Effects of Intravenous Administration of .0 mg/kg HMX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog (10 min and 1 hr)

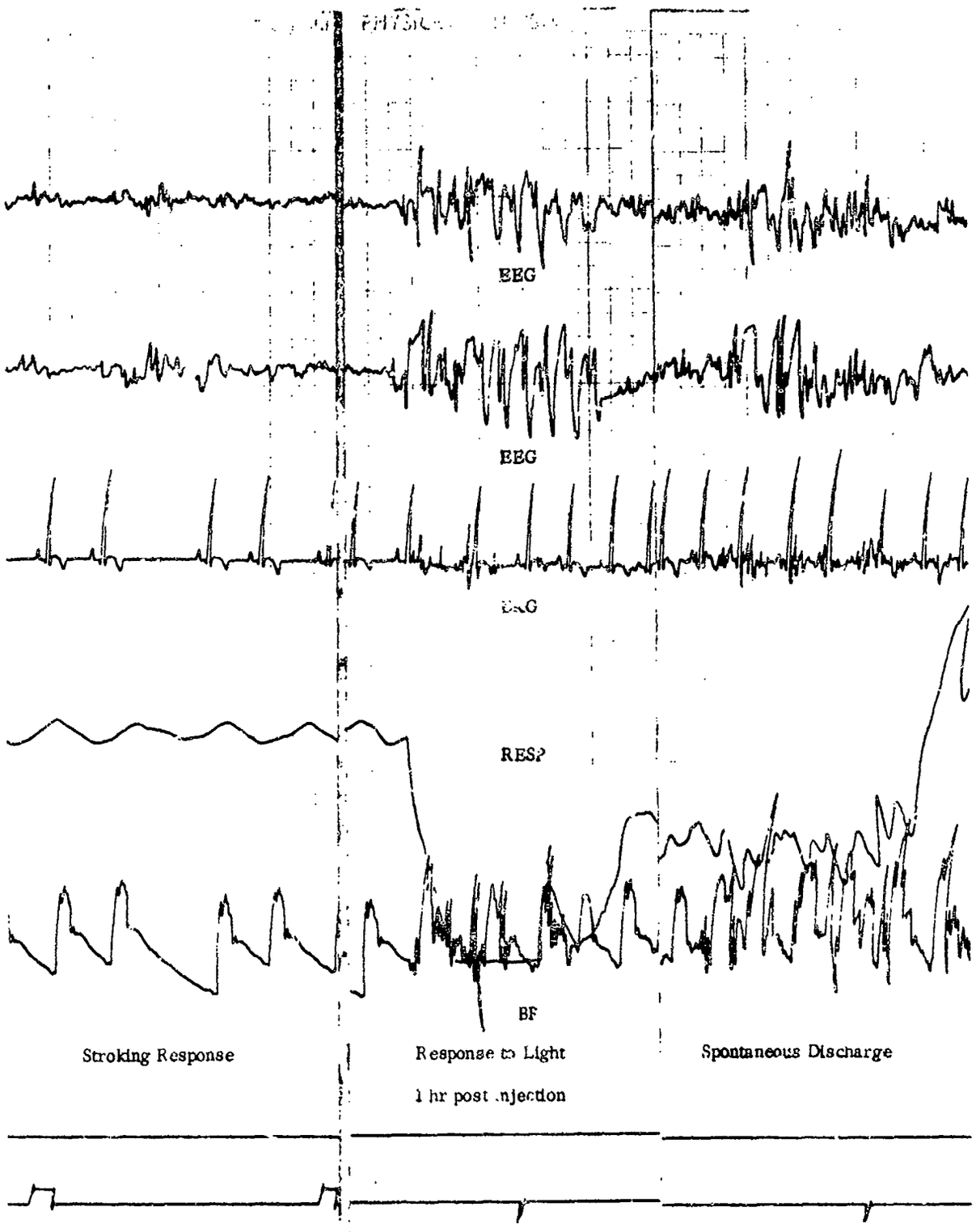

Figure A-18. Effects of Intravenous Administration of 20 mg/kg HMX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog (stroking and light at 1 hr)

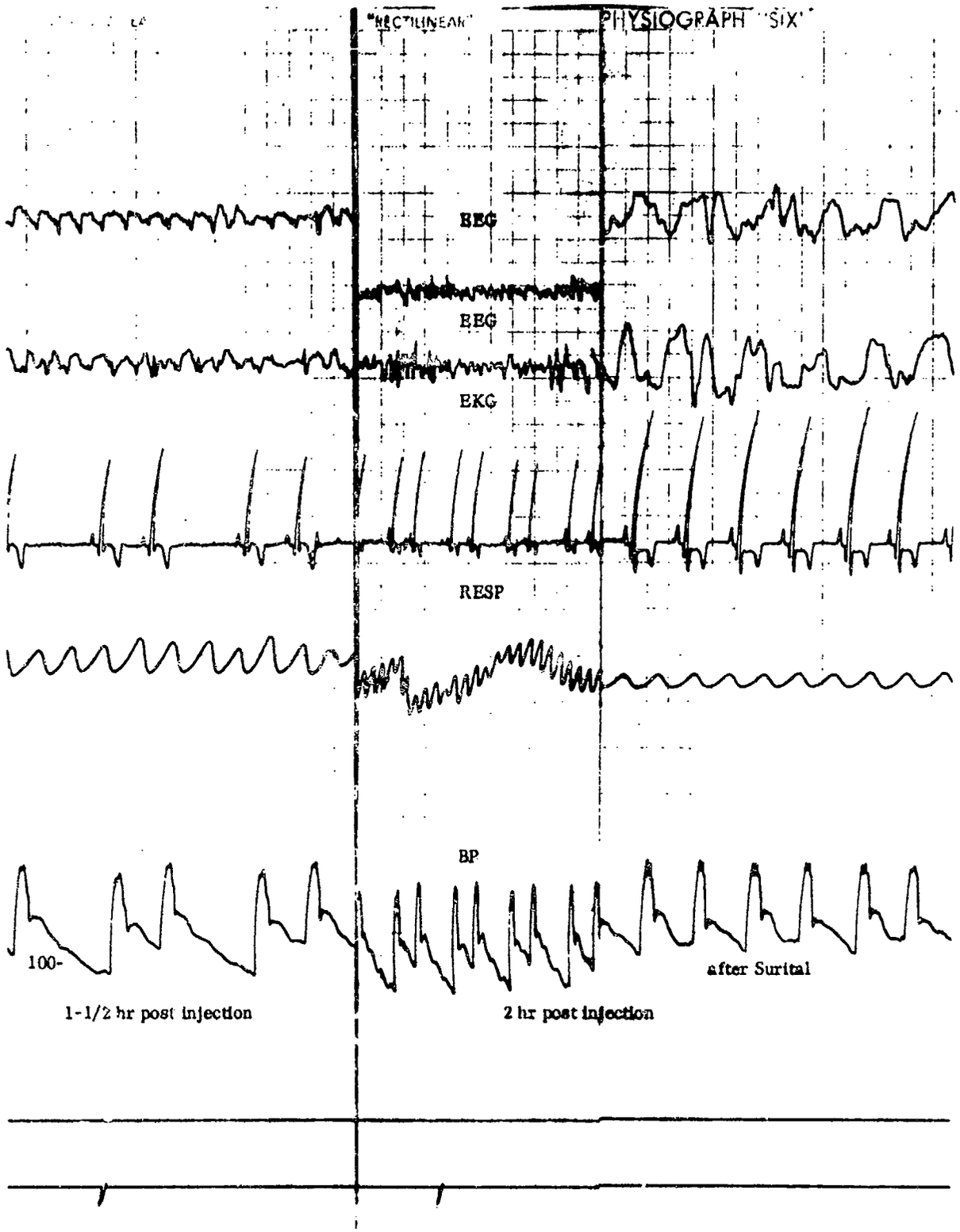

Figure A-19. Effects of Intravenous Administration of 20 mg/kg HMX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog (1-1/2 and 2 hr)

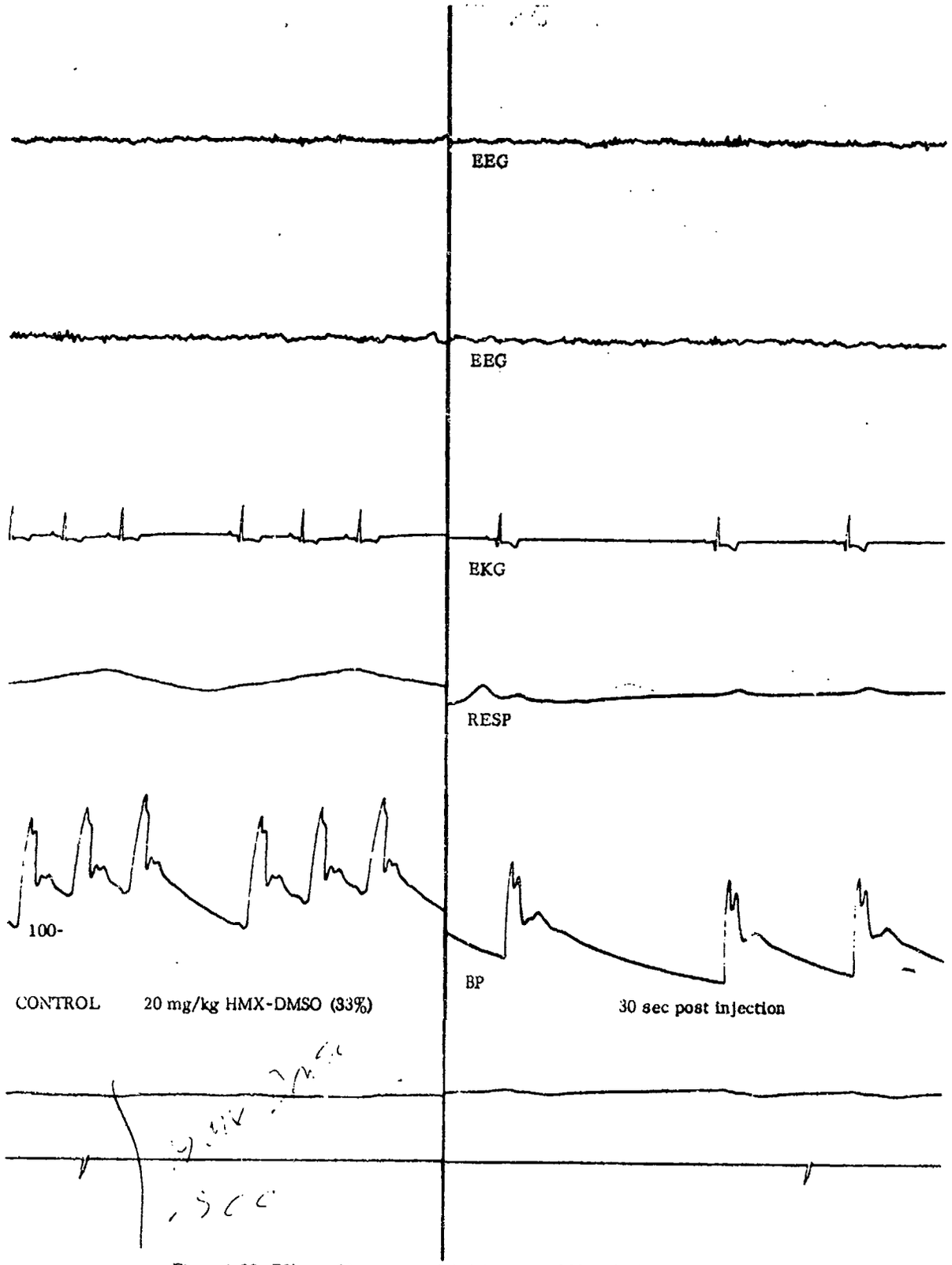

Figure A-20. Effects of Intravenous Administration of 20 mg/kg HMX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog (30 sec)

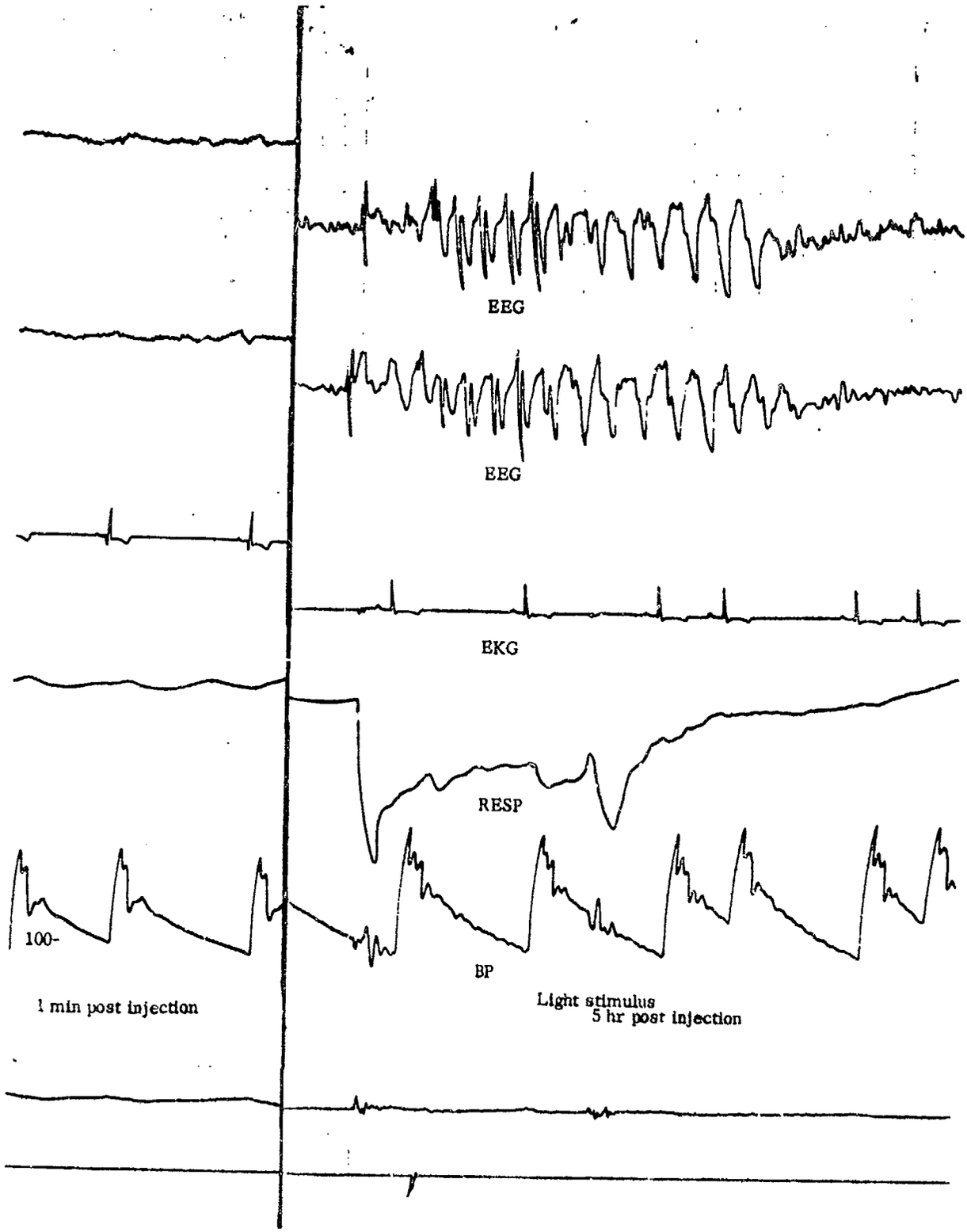

Figure A-21. Effects of Intravenous Administration of 20 mg/kg HMX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog (1 min, 5 hr)

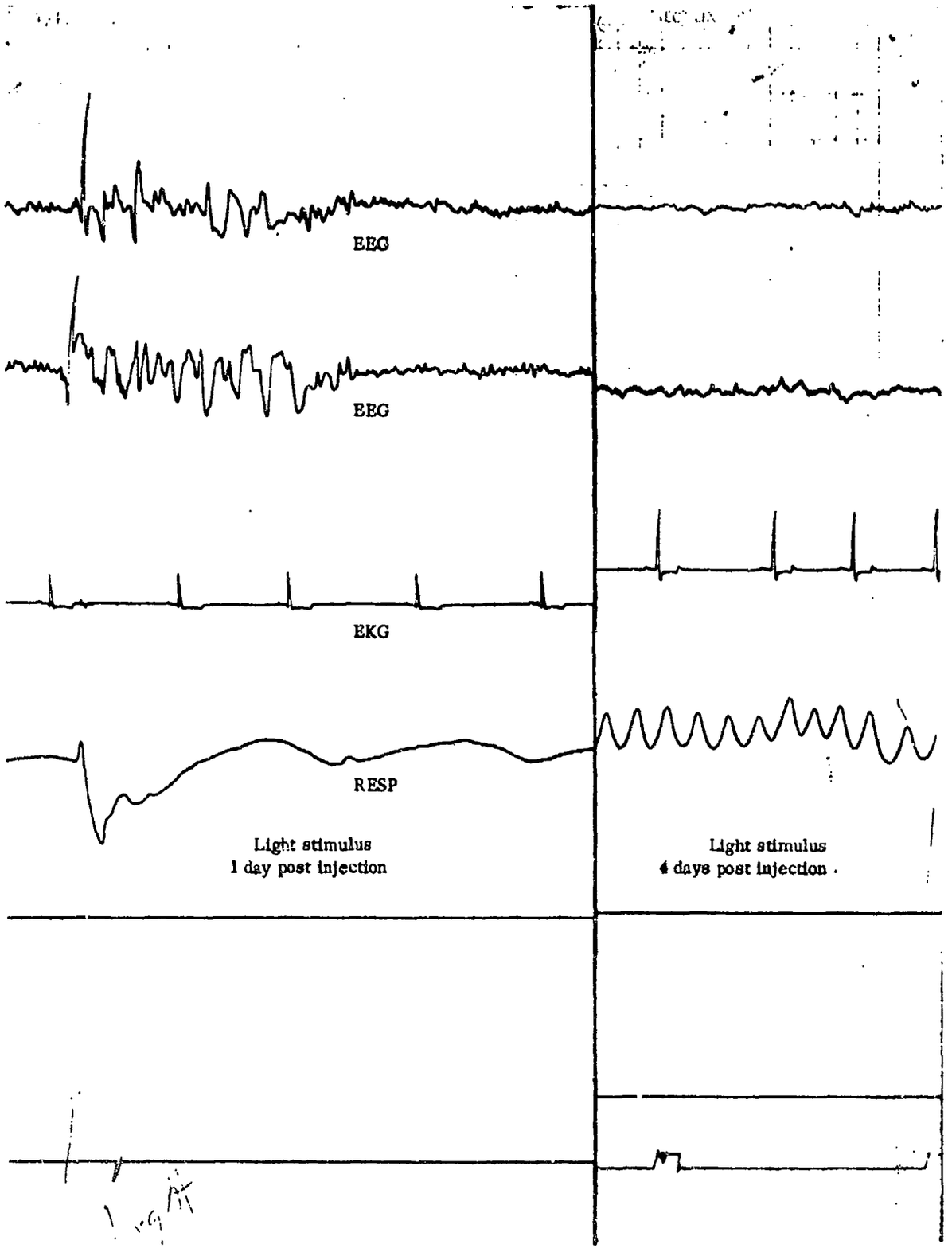

Figure A-22. Effects of Intravenous Administration of 20 mg/kg HMX-DMSO Upon Physiologic Parameters of an Unanesthetized Dog (1 and 4 days)

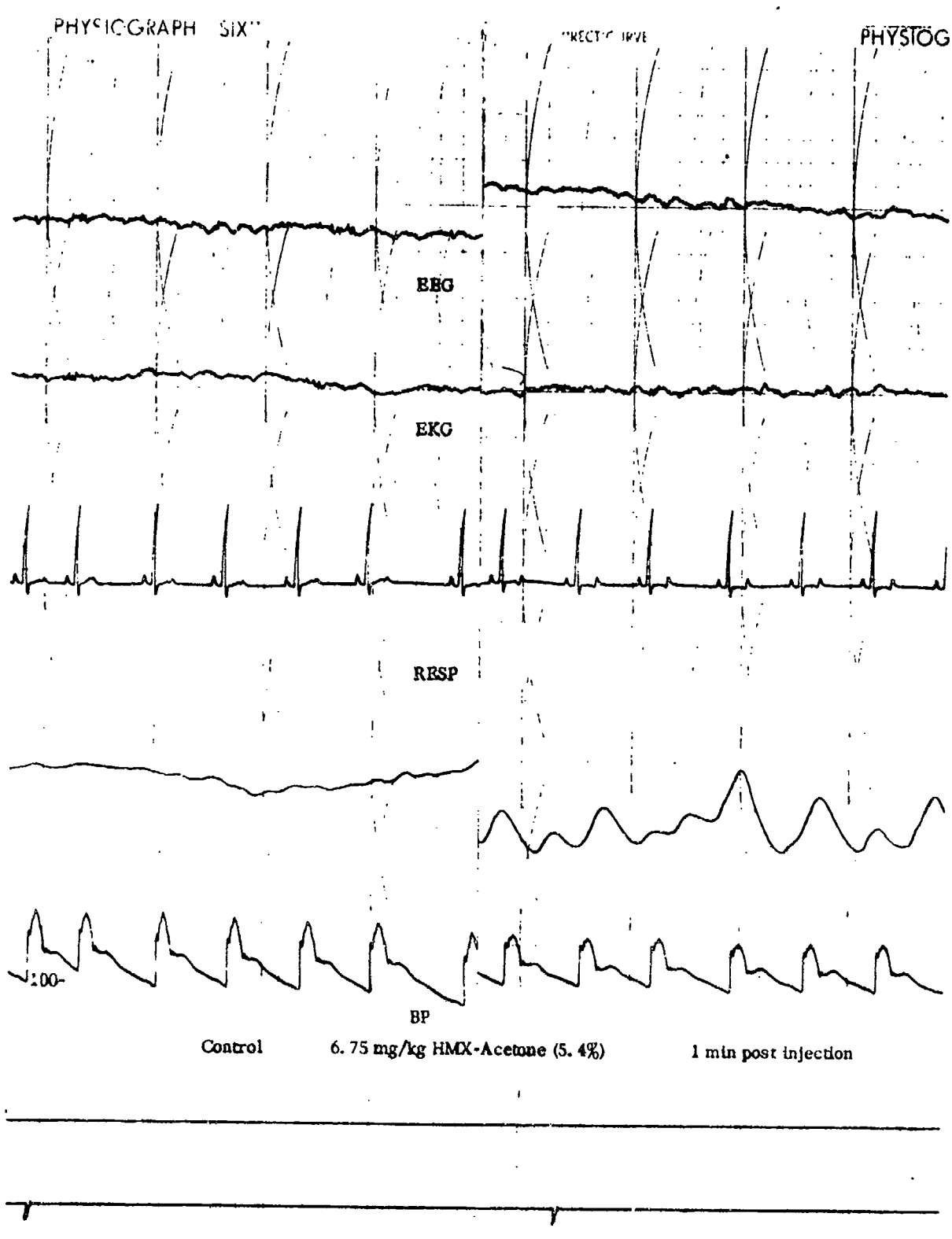

Figure A-23. Effects of Intravenous Administration of 6.75 mg/kg HMX-Acetone Upon Physiologic Parameters of an Unanesthetized Dog (1 min)

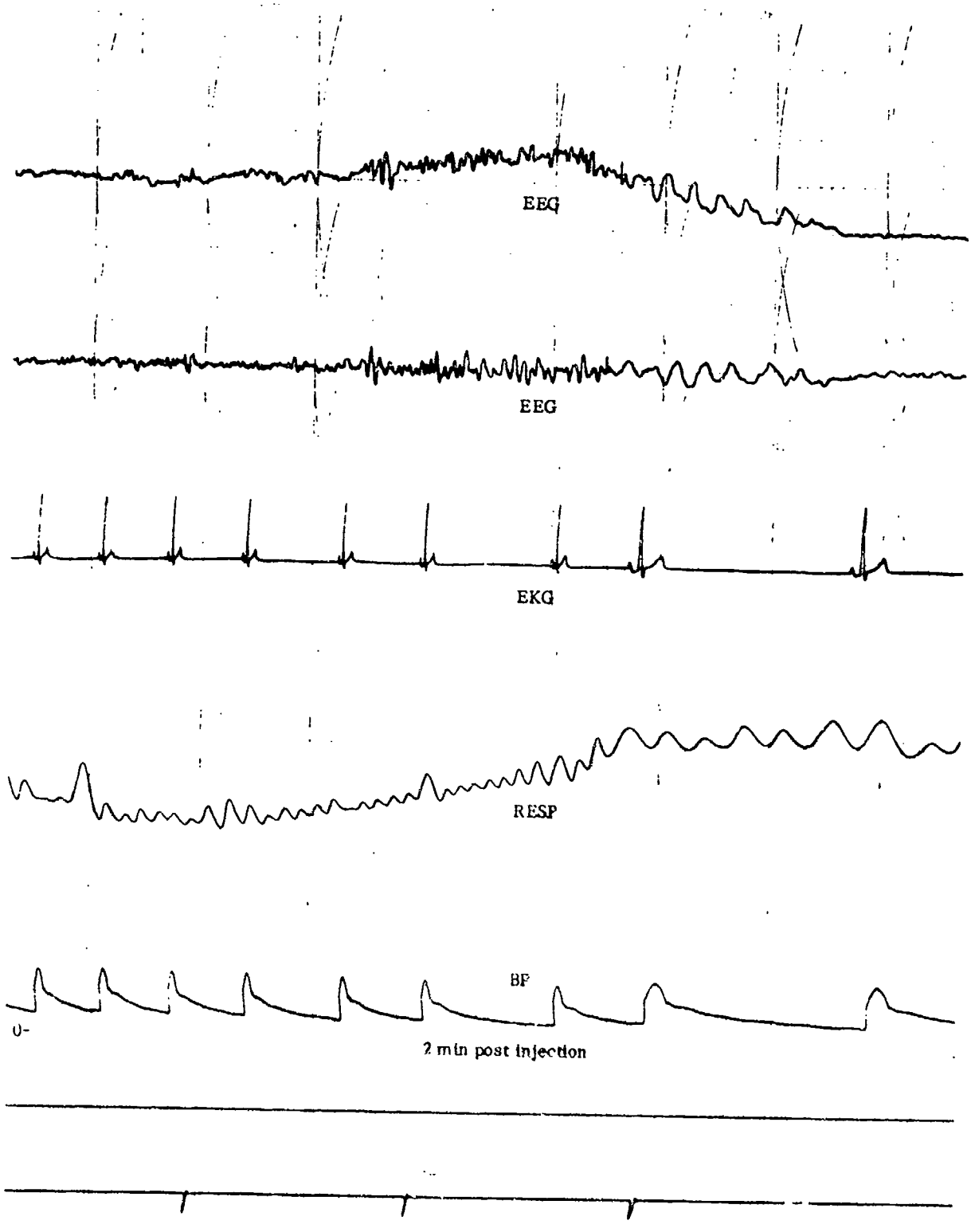

Figure A-24. Effects of Intravenous Administration of 6.75 mg/kg HMX-Acetone Upon Physiologic Parameters of an Unanesthetized Dog (2 min)

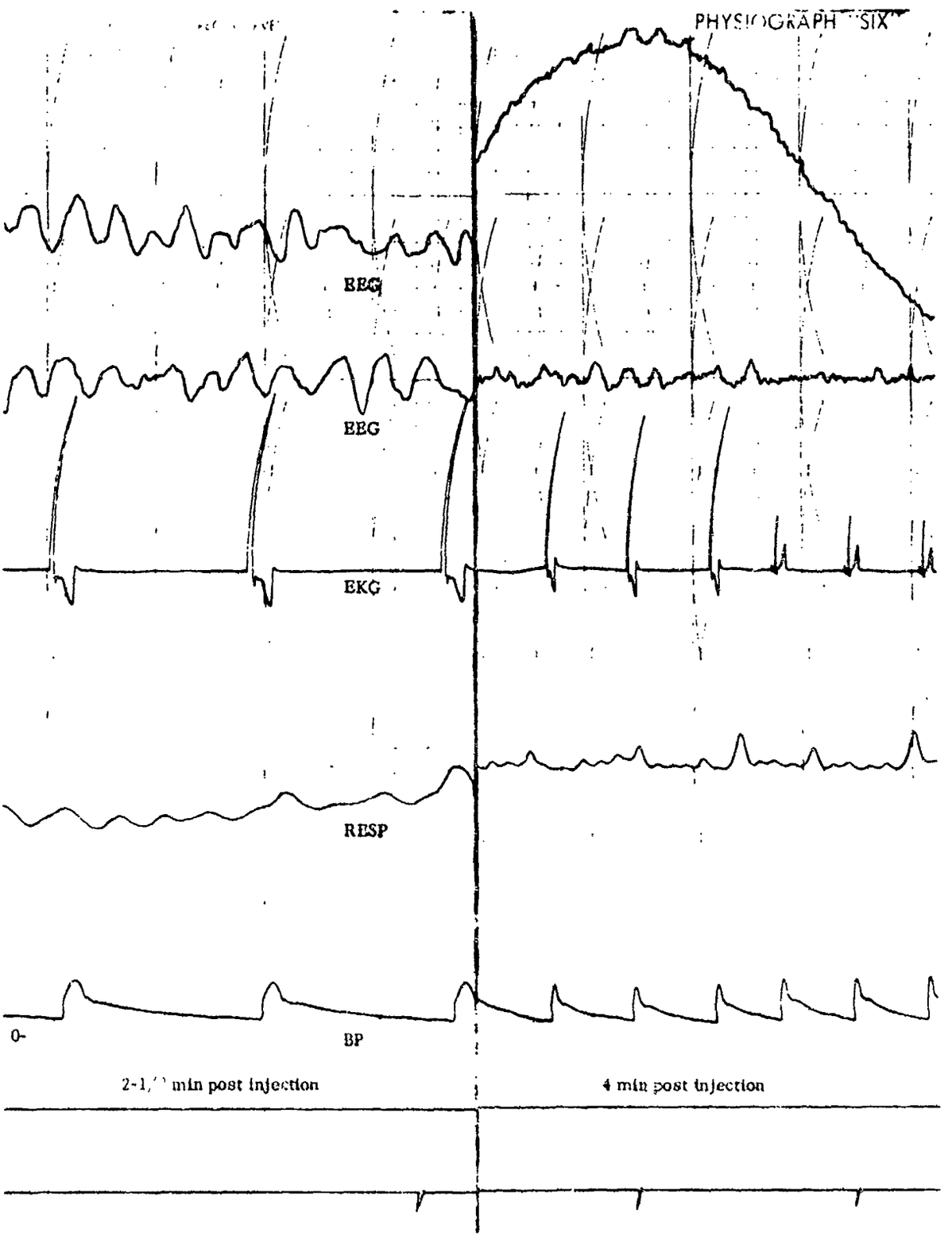

Figure A-25. Effects of Intravenous Administration of 6.75 mg/kg HMX-Acetone Upon Physiologic Parameters of an Unanesthetized Dog (2-1/2 and 4 min)

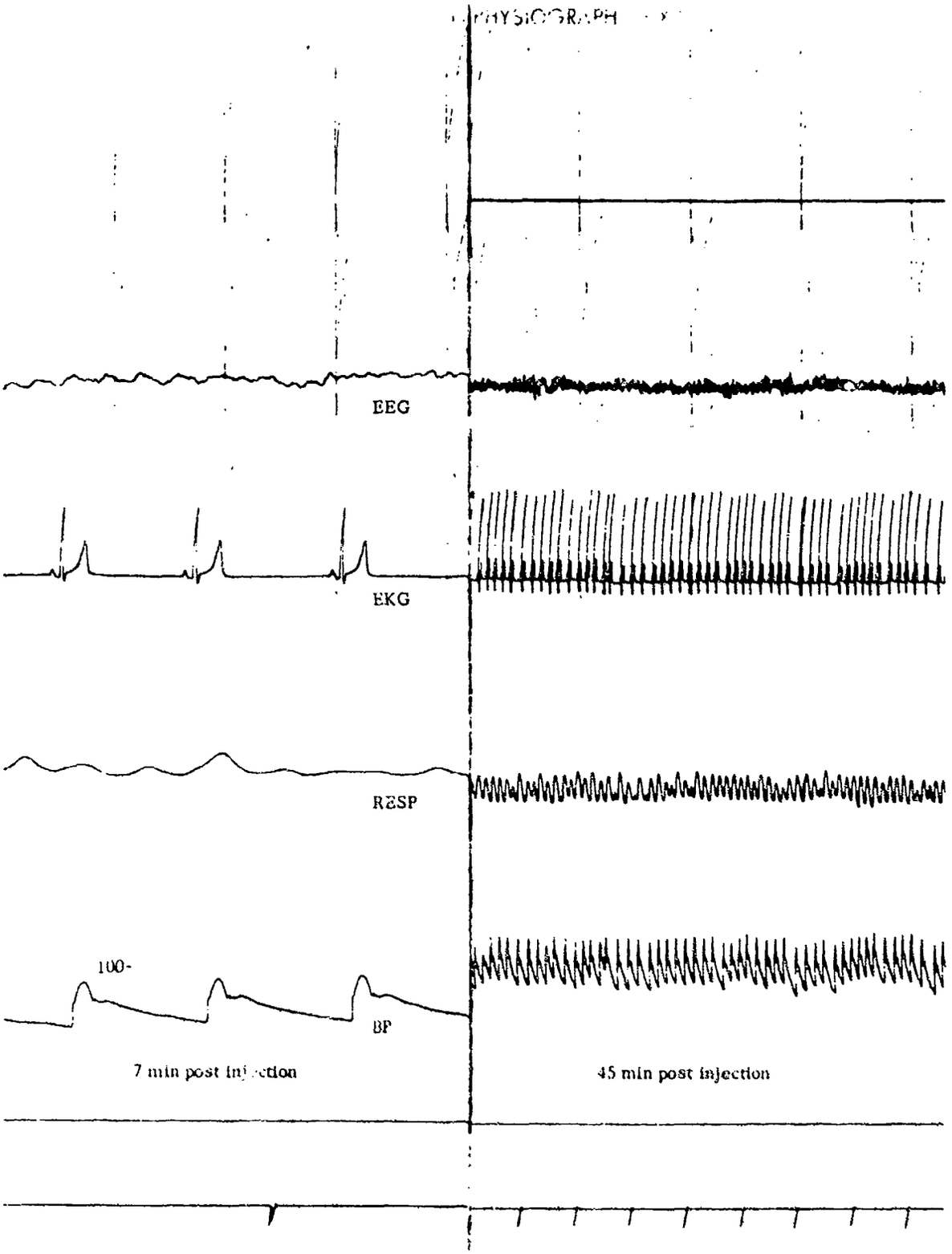

Figure A-26 Effects of Intravenous Administration of 0.75 mg/kg HMX-Acetone Upon Physiologic Parameters of an Unanesthetized Dog (7 and 45 min)

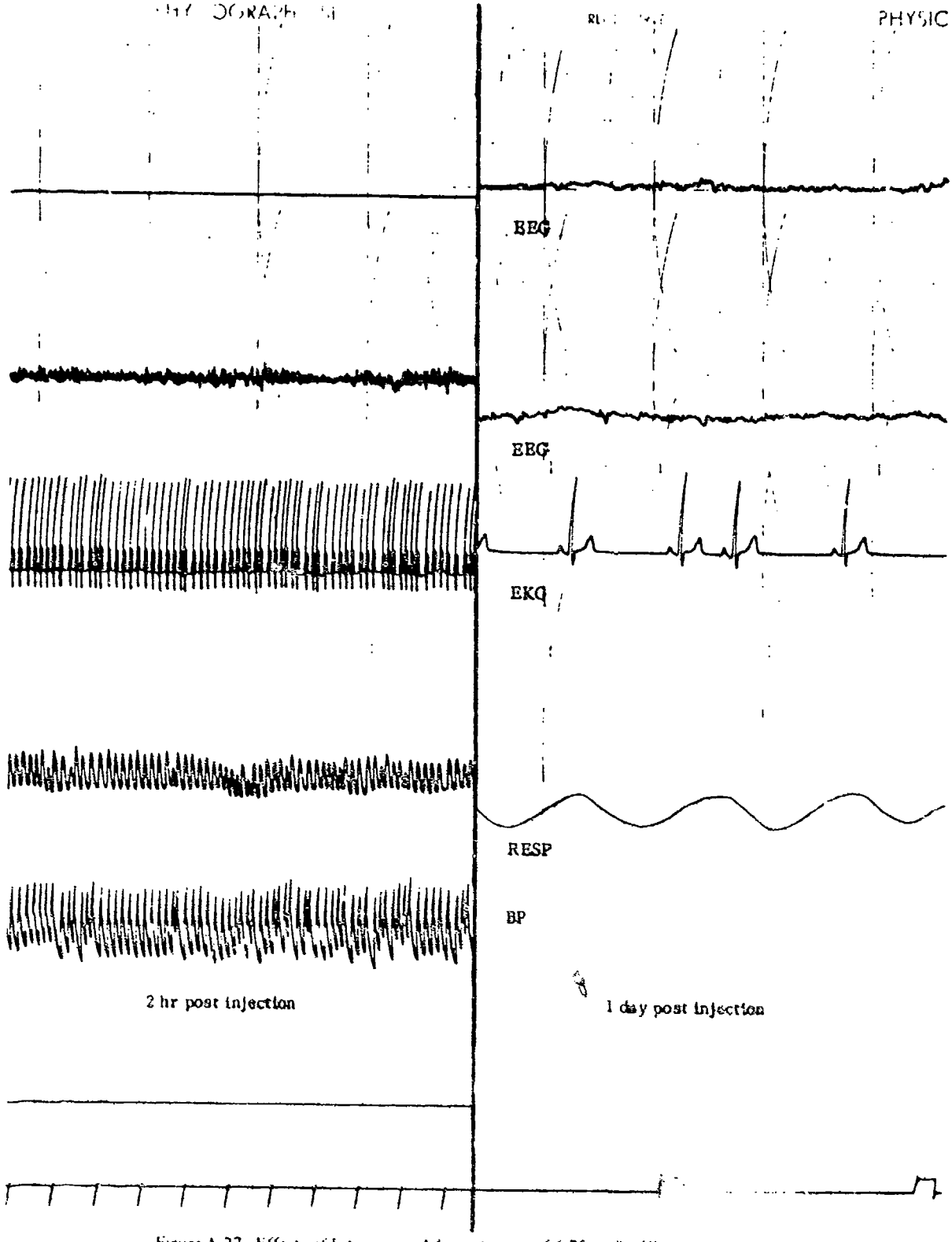

Figure A-27. Effects of Intravenous Administration of 6.75 mg/kg [illegible] pon Physiologic Parameters of an Unanesthetized Dog (2 hr and

APPENDIX B

TABLES

Table B-1. Values for Blood Pressure, Heart Rate, and Respiration During 4-Week Observations in Dogs After Acute Percutaneous Application (1 ml) of RDX, HMX, and Solvents

Treatment	Control			1st Week		2d Week		3d Week		4th Week		
	BP[a]	HR[b]	Resp[c]	HR	Resp	HR	Resp	HR	Resp	BP	HR	Resp
DMSO	180/95	92	16	96	16	104	24	96	30	187/100	114	24
Acetone	175/100	102	12	100	38	108	30	72	50	180/100	120	24
Acetone	190/110	108	12	–	–	120	48	120	–	–	–	–
Cyclo	200/125	156	18	–	–	182	36	120	36	225/100	180	36
RDX-DMSO	187/130	140	14	136	14	96	36	96	36	190/100	114	24
RDX-DMSO	184/120	165	27	104	30	96	24	120	24	180/100	144	26
RDX-Acetone	187/100	144	13	102	24	120	28	84	36	190/100	96	30
RDX-Acetone	190/90	78	30	78	30	72	38	72	48	180/100	84	50
RDX-Cyclo	170/95	144	24	108	24	84	18	108	30	180/100	108	24
RDX-Cyclo	215/125	176	28	110	36	96	36	96	36	165/75	130	30
HMX-DMSO	200/100	156	36	108	48	120	48	168	60	175/75	140	52
HMX-Acetone	180/60	144	24	98	18	108	30	108	36	175/75	84	24
HMX-Cyclo	150/90	102	24	90	54	90	30	84	42	175/100	108	30

[a] BP = femoral arterial blood pressure (mm Hg).

[b] HR = heart rate (beats per minute).

[c] Resp = respiratory rate (breaths per minute).

Table B-II. Pain Threshold and Pupil Response to Light Before and After Acute Percutaneous Application (1 ml) of RDX, HMX, and Solvents

Treatment	Control		1st Week		2d Week		3d Week		4th Week	
	Pain[a]	Pupil response[b]	Pain	Pupil response	Pain	Pupil response	Pain	Pupil response	Pain	Pupil response
	v	mm	v	mm	v	mm	v	mm	v	mm
DMSO	10	–	–	–	10	10.0–3.5	15	10.0–4.0	10	12.5–3.5
Acetone	52	11.5–3.5	37	11.0–3.0	34	11.0–3.0	24	11.0–3.0	30	11.5–3.5
Acetone	25	12.0–3.5	50	11.0–2.5	31	11.0–3.0	–	–	–	–
Cyclo	48	9.0–3.0	–	–	38	10.0–3.5	44	10.5–3.5	28	11.0–2.5
RDX-DMSO	38	10.0–3.5	48	9.5–3.5	40	10.0–2.5	–	–	24	10.0–3.0
RDX-DMSO	32	10.0–4.0	40	10.0–2.0	46	9.5–2.0	40	10.0–2.5	26	10.0–2.0
RDX-Acetone	58	11.0–3.5	45	11.0–3.0	30	12.0–3.5	47	11.5–2.5	–	12.0–3.0
RDX-Acetone	36	–	36	11.5–2.5	37	12.0–2.5	23	9.5–2.0	27	12.0–2.5
RDX-Cyclo	32	9.0–3.0	46	10.0–2.5	34	9.0–2.0	29	11.0–2.0	40	10.0–2.5
RDX-Cyclo	60	11.0–4.0	50	10.0–4.0	60	11.0–4.0	60	10.5–2.5	54	9.5–2.0
HMX-DMSO	60	10.0–2.0	40	10.0–2.5	28	11.0–2.5	26	11.0–2.0	20	9.0–2.0
HMX-Acetone	64	10.0–2.5	33	10.0–3.0	38	10.0–2.5	28	11.0–3.0	20	10.0–2.0
HMX-Cyclo	40	9.0–2.0	31	10.0–2.0	23	9.0–2.0	34	10.0–2.0	22	9.0–2.5

[a] Pain (v) = the amount of voltage needed to elicit a positive response.

[b] Pupil response = the relative size of the pupil before and after light stimulus.

Table B-III. Effects of Certain Test Stimuli Upon Physiologic Parameters After Acute Percutaneous Application (1 ml) of RDX, HMX, and Solvents

Treatment	Nasal stimulation			Vibratory stimulation			Stroking response			Light flash			Auditory response		
	BP	HR	Resp	BP	HR	Resp	BP	HR	Resp	BP	HR	Resp	BP	HR	Resp
DMSO	0	0	↓	0	0	–	–	–	–	0	0	0	–	–	–
Acetone	↓	0	↓	↓	↓	↓	0	0	0	0	0	0	0	0	0
Acetone	0	↓	↓	0	0	0	0	0	0	0	0	0	0	0	0
Cyclo*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RDX-DMSO	0	0	↓	–	–	–	0	0	0	–	–	–	0	0	0
RDX-DMSO	0	↓	↓	–	–	–	0	0	0	0	0	0	–	0	↓
RDX-Acetone	↓	↓	↓	0	↓	0	0		0	–	–	–	0	0	0
RDX-Acetone	–	–	–	0	0	↓	–	–	–	–	–	–	0	0	↓
RDX-Cyclo	↓	↓	↓	↓	↓	↓	↑	0	0	0	0	0	0	0	↓
RDX-Cyclo	–	–	↓	↓	↓	↓	0	0	0	0	↓	0	0	0	0
HMX-DMSO	0	↓	↓	0	↓	↓	0	↓	↓	0	0	0	↓	↓	↓
HMX-Acetone	0	0	↓	0	0	↓	↓	↓	0	–	–	–	↓	↓	↓
HMX-Cyclo	0	↓	↓	0	0	↓	0	↓	↓	0	0	0			

NOTE: 0 = no change
– = not monitored
↑ = increase
↓ = decrease

*Cyclo = cyclohexanone

Table B-IV. Values for Blood Pressure, Heart Rate and Respiration During 4-Week Observations in Dogs After Subacute Percutaneous Application (1 ml/day) of RDX, HMX, and Solvents

Treatment	Control			1st Week		2d Week		3d Week		4th Week		
	BP[a]	HR[b]	Resp[c]	HR	Resp	HR	Resp	HR	Resp	BP	HR	Resp
DMSO	150/100	108	25	96	30	84	24	110	24	130/75	156	30
DMSO	180/115	156	36	144	48	144	120	120	120	175/100	120	90
Acetone	200/100	144	33	108	60	108	36	96	96	175/100	72	48
Acetone	190/100	78	17	102	26	108	36	84	78	190/112	84	40
Cyclo	190/87	120	45	66	60	60	48	84	96	180/100	108	24
Cyclo	200/100	132	26	90	24	90	36	120	70	175/100	126	48
RDX-DMSO	135/90	108	30	96	36	90	18	90	24	190/100	95	80
RDX-DMSO	190/100	112	30	80	30	108	24	108	24	200/100	108	15
RDX-DMSO	–	100	25	100	25	100	25	96	20	–	104	25
RDX-DMSO	–	104	50	120	25	100	24	120	25	–	116	25
RDX-Acetone	190/100	90	84	90	66	108	36	84	78	180/90	84	40
RDX-Acetone	195/100	138	36	126	50	108	48	114	30	190/75	120	42
RDX-Cyclo	180/60	96	26	80	24	72	32	88	40	190/100	84	36
RDX-Cyclo	195/100	138	36	126	50	108	48	114	30	190/75	120	42
HMX-DMSO	180/85	138	33	96	60	120	48	144	75	190/120	102	50
HMX-DMSO	190/112	130	36	84	24	80	36	84	38	165/100	72	36
HMX-DMSO	–	130	40	72	25	96	25	72	25	–	72	25
HMX-DMSO	–	96	60	100	25	90	25	96	25	–	90	25
HMX-Acetone	175/90	100	28	90	36	90	30	96	36	190/100	96	36
HMX-Acetone	185/100	144	24	108	60	120	38	120	38	185/120	84	36
HMX-Cyclo	180/90	132	48	120	24	144	36	156	36	180/100	150	30
HMX-Cyclo	160/75	108	28	100	26	120	24	120	36	180/105	150	24

[a] BP = femoral arterial blood pressure (mm Hg).
[b] HR = heart rate (beats per minute).
[c] Resp = respiratory rate (breaths per minute).

Table B-V. Pain Threshold and Pupil Response to Light Before and After Subacute Percutaneous Application (1 ml/day) of RDX, HMX, and Solvents

Treatment	Control		1st Week		2d Week		3d Week		4th Week	
	Pain[a]	Pupil response[b]	Pain	Pupil response	Pain	Pupil response	Pain	Pupil response	Pain	Pupil response
	v	mm	v	mm	v	mm	v	mm	v	mm
DMSO	48	9.0–1.5	50	9.5–2.0	32	9.0–2.5	41	11.0–2.5	–	–
DMSO	57	11.0–4.0	46	11.0–2.0	38	11.0–3.0	40	10.0–2.5	42	11.0–3.0
Acetone	28	10.0–3.0	18	9.0–3.0	24	11.0–3.0	–	10.0–2.5	–	10.0–2.5
Acetone	62	10.0–3.0	38	10.0–3.0	47	11.0–3.0	42	11.0–3.0	38	10.0–3.0
Cyclo	49	11.0–3.0	–	10.0–2.5	30	11.0–2.5	24	10.5–3.0	24	10.5–3.0
Cyclo	34	11.0–3.5	61	11.0–3.0	43	10.0–2.5	34	10.0–2.0	30	9.0–2.0
RDX-DMSO	47	12.0–3.0	52	11.5–3.5	28	12.0–3.5	26	12.0–3.5	24	11.0–3.5
RDX-DMSO	40	10.0–3.0	55	11.0–3.0	38	11.5–3.5	41	11.0–3.5	20	10.0–3.5
RDX-Acetone	43	11.0–3.0	20	12.0–3.5	–	10.0–2.5	38	11.0–2.5	20	12.0–3.0
RDX-Acetone	37	11.0–3.0	42	10.5–2.5	45	9.5–2.0	44	10.0–2.5	40	10.5–2.5
RDX-Cyclo	34	11.0–4.0	35	9.0–2.5	41	9.0–3.0	44	9.5–3.0	–	–
RDX-Cyclo	52	10.0–2.0	32	9.0–2.0	34	10.0–2.5	24	9.5–2.5	48	10.0–3.0
HMX-DMSO	28	10.0–2.0	15	9.0–2.0	26	9.0–2.0	44	10.0–2.0	35	10.0–3.0
HMX-DMSO	29	10.5–3.5	24	10.0–2.0	40	10.0–2.0	27	9.0–2.0	24	9.0–2.0
HMX-Acetone	40	10.0–3.0	54	10.0–3.0	55	11.0–3.5	49	11.0–3.5	36	11.0–3.0
HMX-Acetone	42	11.0–3.0	20	9.0–2.0	32	10.0–2.5	38	10.0–3.0	38	11.0–3.0
HMX-Cyclo	15	11.0–2.0	–	10.0–2.0	40	9.0–2.0	25	10.0–3.0	31	10.0–3.0
HMX-Cyclo	43	11.0–3.0	50	9.0–2.0	40	10.0–2.0	25	11.0–2.5	33	9.5–3.5

[a]Pain (v) = the amount of voltage needed to elicit a positive response.

[b]Pupil response = the relative size (in mm) of the pupil before and after light stimulus.

Table B-VI. Effects of Certain Test Stimuli Upon Physiologic Parameters After Subacute Percutaneous Application (1 ml/day) of RDX, HMX, and Solvents

Treatment	Nasal stimulation			Vibratory stimulation			Stroking response			Light flash			Auditory response		
	BP	HR	Resp	BP	HR	Resp	BP	HR	Resp	BP	HR	Resp	BP	HR	Resp
DMSO	0	↓	↓	0	↓	↓	0	0	↓	0	0	↓	0	0	↓
DMSO	↓	↓	↓	↓	↓	↓	–	–	–	↓	↓	↓	0	↓	0
Acetone	0	↓	↓	0	0	0	–	–	–	0	0	↓↑	0	0	↓↑
Acetone	0	0	↓↑	0	0	↓↑	–	–	–	0	↓	↓	↓	↓	↓
Cyclo*	0	0	↓	0	↓	↓	0	0	0	0	0	↓↑	0	↓	0
Cyclo	0	0	↓	0	0	↓	–	–	–	↓	↓	↓	↓	↓	↓
RDX-DMSO	0	0	↓	0	↓	0	0	0	0	0	0	0	0	0	0
RDX-DMSO	0	↓	↓	0	0	↓	0	0	0	0	↓	↓	–	–	–
RDX-DMSO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
RDX-DMSO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
RDX-Acetone	↓	↓	↓	0	↓	0	0	↓	↓	0	↓	↓	0	0	↓
RDX-Acetone	0	0	↓	0	↓	0	↓	↓	↓	0	↓	0	0	↓	↓
RDX-Cyclo	0	0	↓	0	0	↓↑	–	–	–	0	0	↓	0	0	0
RDX-Cyclo	0	↓	↓	0	↓	↓	0	0	↓↑	0	0	↓	0	↓	↓
HMX DMSO	0	↓	↓	0	0	↓	0	0	0	0	↓	↓↑	0	↓	↓
HMX-DMSO	0	↓	↓	0	0	↓↑	0	0	0	0	↓	0	0	↓	↓
HMX-DMSO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HMX-DMSO	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HMX-Acetone	0	↓	↓	0	↓	↓	0	0	↓↑	0	↓	↓↑	0	0	↓↑
HMX-Acetone	0	↓	↓	0	↓	↓	0	0	↓↑	0	0	↓↑	0	↓	↓
HMX-Cyclo	0	0	↓	0	0	↓↑	0	0	↓↑	0	0	↓↑	0	↓	0
HMX-Cyclo	0	↓	↓	0	↓	↓	0	0	0	0	↓	↓	0	↓	↓

NOTE: 0 = no change
– = not monitored
↑ = increase
↓ = decrease

*Cyclo = cyclohexanone.

Table B-VII. The Sensitization Potential in Clipped Guinea Pigs of Intradermally Administered RDX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO When Challenged by the Percutaneous Route

Sensitization exposure[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Solvent and dilution	4–5 hr	24 hr	48 hr	78 hr
	%		days						
RDX	0.25	1:1 Acetone-saline	80	RDX–5.4% acetone	1:10 PEG	0	0	0	0
RDX	0.25	1:1 Cyclohexanone-saline	80	RDX–7.5% cyclohexanone	1:10 PEG	0	0	0	0
RDX	0.25	1:1 Tech grade DMSO-saline	80	RDX–33% tech grade DMSO	1:10 PEG	0	0	0	0
Acetone	0.50	Saline	80	RDX–5.4% acetone	1:10 PEG	0	0	0	0
Cyclohexanone	0.50	Saline	80	RDX–7.5% cyclohexanone	1:10 PEG	0	0	0	0
Pure DMSO	0.50	Saline	80	RDX–33% pure DMSO	1:10 PEG	0	0	0	0
Tech grade DMSO	0.50	Saline	80	RDX–33% tech grade DMSO	1:10 PEG	0	0	0	0

[a] 0.05 Ml given intradermally (dorsal thorax) 3 times per week for 3 weeks to six animals.

[b] 0.5 Ml given topically (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27).

Table B-VIII. The Sensitization Potential in Clipped Guinea Pigs of Intradermally Administered RDX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO as Determined by Intradermal Challenge

Sensitization exposure[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Solvent and dilution	6 hr	24 hr	48 hr	72 hr
	%		days						
RDX	0.25	1:1 Acetone-saline	18	RDX-0.25% 1:1 acetone-saline	1:64 Saline	1-E1	1-E1	0	0
RDX	0.25	1:1 Cyclohexanone-saline	18	RDX-0.25% 1:1 cyclohexanone-saline	1:64 Saline	2-E1	0	0	0
RDX	0.25	1:1 Tech grade DMSO-saline	18	RDX-0.25% 1:1 tech grade-saline	1:64 Saline	0	0	0	0
Acetone	0.5	Saline	18	RDX-0.25% 1:1 acetone-saline	1:64 Saline	0	0	0	0
Cyclohexanone	0.5	Saline	18	RDX-0.25% 1:1 cyclohexanone-saline	1:64 Saline	1-E1	1-E1	0	0
Pure DMSO	0.5	Saline	18	RDX-0.25% 1:1 tech grade DMSO-saline	1:64 Saline	0	0	0	0
Tech grade DMSO	0.5	Saline	18	RDX-0.25% 1:1 tech grade DMSO-saline	1:64 Saline	1-E1	1-E1	0	0

[a] 0.05 Ml given intradermally (dorsal thorax) 3 times per week for 3 weeks to six animals.

[b] 0.05 Ml given intradermally (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27)

Table B-IX The Sensitization Potential in Clipped Guinea Pigs of Percutaneously Applied RDX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO as Determined by Topical Challenge Using Polyethylene Glycol 200

Sensitization exposure[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Solvent and dilution	5 hr	24 hr	48 hr	72 hr
	%		days						
RDX	5.4	Acetone	25	RDX-5.4% acetone	1:10 PEG	0	0	0	0
RDX	7.5	Cyclohexanone	25	RDX-7.5% cyclohexanone	1:10 PEG	0	0	0	0
RDX	33.0	Pure DMSO	25	RDX-33% pure DMSO	1:10 PEG	0	0	0	0
RDX	33.0	Tech grade DMSO	25	RDX-33% tech grade DMSO	1:10 PEG	0	0	0	0
Acetone	100.0	—	25	RDX-5.4% acetone	1:10 PEG	0	0	0	0
Cyclohexanone	100.0	—	25	RDX-7.5% cyclohexanone	1:10 PEG	0	0	0	0
Pure DMSO	100.0	—	25	RDX-33% pure DMSO	1:10 PEG	0	0	0	0
Tech grade DMSO	100.0	—	25	RDX-33% tech grade DMSO	1:10 PEG	0	0	0	0

[a] 0.5 Ml given topically (dorsal thorax) 3 times per week for 3 weeks to six animals.

[b] 0.5 Ml given topically (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27).

Table B-X The Sensitization Potential in Clipped Guinea Pigs of Percutaneously Applied RDX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO as Determined by Intradermal Challenge

Sensitization exposures[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Solvent and dilution	4 hr	24 hr	48 hr	72 hr
	%		days						
RDX	5.4	Acetone	18	RDX-0.25% 1:1 acetone and saline	1:32	0	0	0	0
RDX	7.4	Cyclohexanone	18	RDX-0.25% 1:1 cyclohexanone and saline	1:64	0	0	0	0
RDX	33.0	Pure DMSO	18	RDX-0.25% 1:1 pure DMSO-saline	1:32	0	0	0	0
RDX	33.0	Tech grade DMSO	18	RDX-0.25% 1:1 tech grade DMSO saline	1:32	0	0	0	0
Acetone	100.0	—	18	RDX-0.25% 1:1 acetone and saline	1:32	0	1-E1	0	0
Cyclohexanone	100.0	—	18	RDX-0.25% 1:1 cyclohexanone and saline	1:64	0	0	0	0
Pure DMSO	100	—	18	RDX-0.25% 1:1 pure DMSO-saline	1:32	0	0	0	0
Tech grade DMSO	100.0	—	18	RDX-0.25% 1:1 tech grade DMSO-saline	1:32	0	0	0	0

[a] 0.5 Ml given topically (dorsal thorax) 3 times per week for 3 weeks to six animals.

[b] 0.05 Ml saline given intradermally (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27).

Table B-XI The Sensitization Potential in Clipped Guinea Pigs of Intradermally Administered HMX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO When Challenged by the Percutaneous Route

Sensitization exposures[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Solvent and dilution	5–6 hr	24 hr	48 hr	72 hr
	%		days						
HMX	0.25	1:1 Acetone-saline	25	HMX–2% acetone	1:10 PEG	0	0	0	0
HMX	0.25	1:1 Cyclohexanone-saline	25	HMX–2.5% cyclohexanone	1:10 PEG	0	0	0	0
HMX	0.25	1:1 Pure DMSO-saline	25	HMX–3.3% pure DMSO	1:10 PEG	0	0	0	0
HMX	0.25	1:1 Tech grade DMSO-saline	25	HMX–3.3% tech grade DMSO	1:10 PEG	0	0	0	0
Acetone	0.50	Saline	25	HMX–2% acetone	1:10 PEG	0	0	0	0
Cyclohexanone	0.50	Saline	25	HMX–2.5% cyclohexanone	1:10 PEG	0	0	0	0
Pure DMSO	0.50	Saline	25	HMX–3.3% pure DMSO	1:10 PEG	0	0	0	0
Tech grade DMSO	0.50	Saline	25	HMX 3.3% tech grade DMSO	1:10 PEG	0	0	0	0

[a] 0.05 Ml given intradermally (dorsal thorax) 3 times per week for 3 weeks to six animals.

[b] 0.5 Ml PEG given topically (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27).

Table B-XII. The Sensitization Potential in Clipped Guinea Pigs of Intradermally Administered HMX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO as Determined by Intradermal Challenge

Sensitization exposures[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Solvent and dilution	5–6 hr	24 hr	48 hr	72 hr
	%		days						
HMX	0.25	1:1 Acetone-saline	20	HMX-0.25% 1:1 acetone-saline	1:32	0	0	0	0
HMX	0.25	1:1 Cyclohexanone-saline	20	HMX-0.25% 1:1 cyclohexanone-saline	1:64	0	0	0	0
HMX	0.25	1:1 Pure DMSO-saline	20	HMX-0.25% 1:1 pure DMSO-saline	1:32	0	0	0	0
HMX	0.25	1:1 Tech DMSO-saline	20	HMX-0.25% 1:1 tech DMSO-saline	1:32	0	0	0	0
Acetone	0.50	Saline	20	HMX-0.25% 1:1 acetone-saline	1:32	0	0	0	0
Cyclohexanone	0.50	Saline	20	HMX-0.25% 1:1 cyclohexanone-saline	1:64	0	0	0	0
Pure DMSO	0.50	Saline	20	HMX-0.25% 1:1 pure DMSO-saline	1:32	0	0	0	0
Tech grade DMSO	0.50	Saline	20	HMX-0.25% 1:1 tech DMSO-saline	1:32	0	0	0	0

[a] 0.05 Ml given intradermally (dorsal thorax) 3 times per week for 3 weeks to six animals.

[b] 0.05 Ml saline given intradermally (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27).

Table B-XIII The Sensitization Potential in Clipped Guinea Pigs of Percutaneously Applied HMX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO as Determined by Percutaneous Challenge Using Polyethylene Glycol 200

Sensitization exposures[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Solvent and dilution	6 hr	24 hr	48 hr	72 hr
	%		days						
HMX	2.0	Acetone	25	HMX-2.0% acetone	1:10 PEG	0	0	0	0
HMX	2.5	Cyclohexanone	25	HMX-2.5% cyclohexanone	1:10 PEG	0	0	0	0
HMX	3.3	Pure DMSO	25	HMX-3.3% pure DMSO	1:10 PEG	0	0	0	0
HMX	33.0	Pure DMSO	25	HMX-33.0% pure DMSO	1:10 PEG	0[d]	0[d]	0[d]	0[d]
HMX	3.3	Tech grade DMSO	25	HMX-3.3% tech grade DMSO	1:10 PEG	0	0	0	0
HMX	33.0	Tech grade DMSO	25	HMX-33.0% tech grade DMSO	1:10 PEG	0[d]	0[d]	0[d]	0[d]
Acetone	100.0	—	25	HMX-2.0% acetone	1:10 PEG	0	0	0	0
Cyclohexanone	100.0	—	25	HMX-2.5% cyclohexanone	1:10 PEG	0	0	0	0
Pure DMSO	100.0	—	25	HMX-3.3% pure DMSO	1:10 PEG	0	0	0	0
Tech. grade DMSO	100.0	—	25	HMX-3.3% tech grade DMSO	1:10 PEG	0	0	0	0

[a] 0.5 Ml given topically (dorsal thorax) 3 times per week for 3 weeks to six animals.

[b] 0.5 Ml PEG given topically (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27).

[d] Two animals from the 33% HMX/pure DMSO group and three animals from the 33% HMX/tech grade DMSO group died during sensitizing process.

Table B-XIV. The Sensitization Potential in Clipped Guinea Pigs of Percutaneously Applied HMX in Acetone, Cyclohexanone, and Pure and Technical Grade DMSO as Determined by Intradermal Challenge

Sensitization exposures[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Dilution	6 hr	24 hr	48 hr	72 hr
	%		days						
HMX	2.0	Acetone	18	HMX-0.25% 1:1 acetone-saline	1:32	0	0	0	0
HMX	2.5	Cyclohexanone	18	HMX-0.25% 1:1 cyclohexanone-saline	1:64	0	0	0	0
HMX	3.3	Pure DMSO	18	HMX-0.25% 1:1 pure DMSO-saline	1:32	0	0	0	0
HMX	33.3	Pure DMSO	18	HMX-0.25% 1:1 pure DMSO-saline	1:32	0[d]	0[d]	0[d]	0[d]
HMX	3.3	Tech grade DMSO	18	HMX-0.25% 1:1 tech grade DMSO-saline	1:32	0	0	0	0
HMX	33.3	Tech grade DMSO	18	HMX-0.25% 1:1 tech grade DMSO-saline	1:32	0[d]	0[d]	0[d]	0[d]
Acetone	100.0	—	18	HMX-0.25% 1:1 acetone-saline	1:32	0	0	0	0
Cyclohexanone	100.0	—	18	HMX-0.25% 1:1 cyclohexanone-saline	1:64	0	0	0	0
Pure DMSO	100.0	—	18	HMX-0.25% 1:1 pure DMSO-saline	1:32	0	0	0	0
Tech grade DMSO	100.0	—	18	HMX-0.25% 1:1 tech grade DMSO-saline	1:32	0	0	0	0

[a] 0.5 Ml given topically (dorsal thorax) 3 times per week for 3 weeks to six animals.

[b] 0.5 Ml saline given intradermally (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27).

[d] Two animals from the 33% HMX/pure DMSO group and three animals from the 33% HMX/tech grade DMSO group died during sensitizing process.

Table B-XV. The Sensitization Potential in Clipped Guinea Pigs of Intradermally Administered Acetone, Cyclohexanone, and Pure and Technical Grade DMSO When Challenged by the Percutaneous Route

Sensitization exposures[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Solvent and dilution	4 hr	24 hr	48 hr	72 hr
	%		days						
Acetone	50	1:1 Acetone-saline	24	Acetone	1:10 PEG	0	0	0	0
Cyclohexanone	50	1:1 Cyclohexanone-saline	24	Cyclohexanone	1:10 PEG	0	0	0	0
Pure DMSO	50	1:1 Pure DMSO-saline	24	Pure DMSO	1:10 PEG	0	0	0	0
Tech grade DMSO	50	1:1 Tech grade DMSO-saline	24	Tech grade DMSO	1:10 PEG	0	0	0	0

[a] 0.05 Ml given intradermally (dorsal thorax) 3 times per week for 3 weeks to four animals.

[b] 0.5 Ml PEG given topically (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27).

Table B-XVI The Sensitization Potential in Clipped Guinea Pigs of Intradermally Administered Acetone, Cyclohexanone, and Pure and Technical Grade DMSO as Determined by Intradermal Challenge

Sensitization exposures[a]			Rest period	Challenge exposure[b]		Skin effects[c]			
Compound	Strength of solution	Solvent		Compound	Solvent and dilution	6 hr	24 hr	48 hr	72 hr
	%		days						
Acetone	50	1:1 Acetone-saline	17	Acetone 50% 1:1 saline	1:64 saline	0	0	0	0
Cyclohexanone	50	1:1 Cyclohexanone-saline	17	Cyclohexanone 50% 1:1 saline	1:64 saline	0	0	0	0
Pure DMSO	50	1:1 Pure DMSO-saline	17	Pure DMSO 50% 1:1 saline	1:64 saline	0	0	0	0
Tech grade DMSO	50	1:1 Tech grade DMSO-saline	17	Tech grade DMSO 50% 1:1 saline	1:64 saline	0	0	0	0

[a] 0.05 Ml given intradermally (dorsal thorax) 3 times per week for 3 weeks to four animals.

[b] 0.05 Ml given intradermally (thigh).

[c] The Draize test was used to evaluate skin effects (table VII, p. 27).